Cloning

Recombinat DNA Technology or Genetic Engineering

In the 1950s and 60s, the Biologists used biochemical, biophysical and crystallographic procedures to discover the double helix structure of DNA. But, they were frustrated not being able to examine a small fragment of DNA such as a gene in pure and isolated form to perform critical experiments to discover the detail working of a gene. This situation led to the discovery of the the recombinant DNA technology or genetic engineering in the 1970s. At the core of this new technology was gene cloning. In order to study a specific fragment of DNA sequence, we need to select the fragment and *amplify* it so that the solution contains a purified near-homogeneous population.

The basic steps of the gene cloning experiment is as follows:

1. A fragment of DNA containing the gene to be cloned is inserted into second(usuallyCircular) DNA molecule called a **vector.**

2. The recombinant molecule is inserted into a host cell by a procedure called **transformation**.

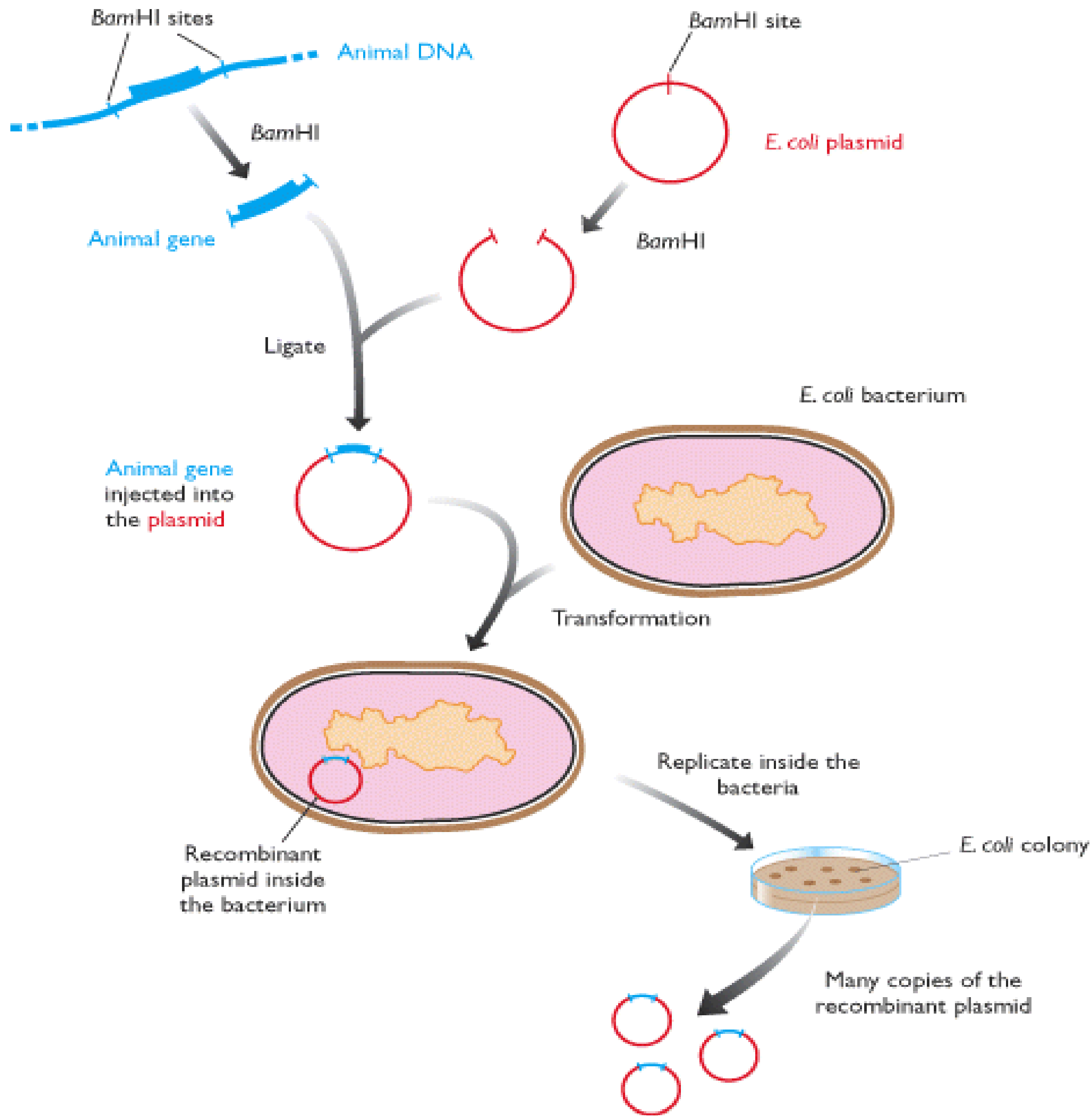
3. Within the host cell the vector multiplies producing a number of identical copies of the recombinant DNA molecule.

4. When the host cell divides , the copies of the recombinant molecule is passed on to the to the progeny and further replication of the recombinant molecule takes place.

5. A large number of cell divisions give rise to a clone, a colony or culture of the recombinant molecule .

A naturally occurring vector is a ***plasmid*** which is a circular DNA found in bacteria. Plasmids can infect bacteria such as *E.Coli*. Cutting plasmids with a **restriction enzyme** that has also been used to cut the DNA creating compatible sticky end.
This allows formation of **recombinant plasmids**. The resulting molecule is then inserted into a suitable host (a bacteria or yeast cell) and the organism multiply under suitable conditions (temperature and nutrients), producing a colony of identical cell clones. The host is then killed and the resulting DNA pieces extracted and sequenced. The method is explained in the following slide:

DNA cloning



Why Cloning is so significant?

Purity: The DNA fragment may be in a mixture of many different fragments, each carrying different genes different lengths. All these genes will be Inserted in different vectors and each of these vectors will keep multiplying In the dish. Only one of these carries the gene of interest and multiple copies of the recombinant molecule will be present in the culture. In the laboratory, The biologists have developed techniques to extract only the vectors of only one recombinant molecule and retrive the gene out of it.

The technology is now extensively used in genetic engineering of plants and anima;s, cancer research and stem cell research.

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Vectors for cloning vary depending on the size of the DNA to be cloned. There are many types of cloning vectors available allowing varying sizes of DNA inserts to be amplified. The table below gives a partial list. This includes plasmids, viruses, yeast artificial chromosomes (YAC) and bacterial artificial chromosome (BAC) which were used to create overlapping clones for sequencing human genome.

Cloning Vectors

Cloning Vector	Insert Size
Bacteriophage M13	1.5 kb
Plasmid	5 kb
Bacteriophage λ	25 kb
Cosmid	40 kb
BAC(bacterial artificial chromosome)	150 kb
YAC(yeast artificial chromosome)	500 kb