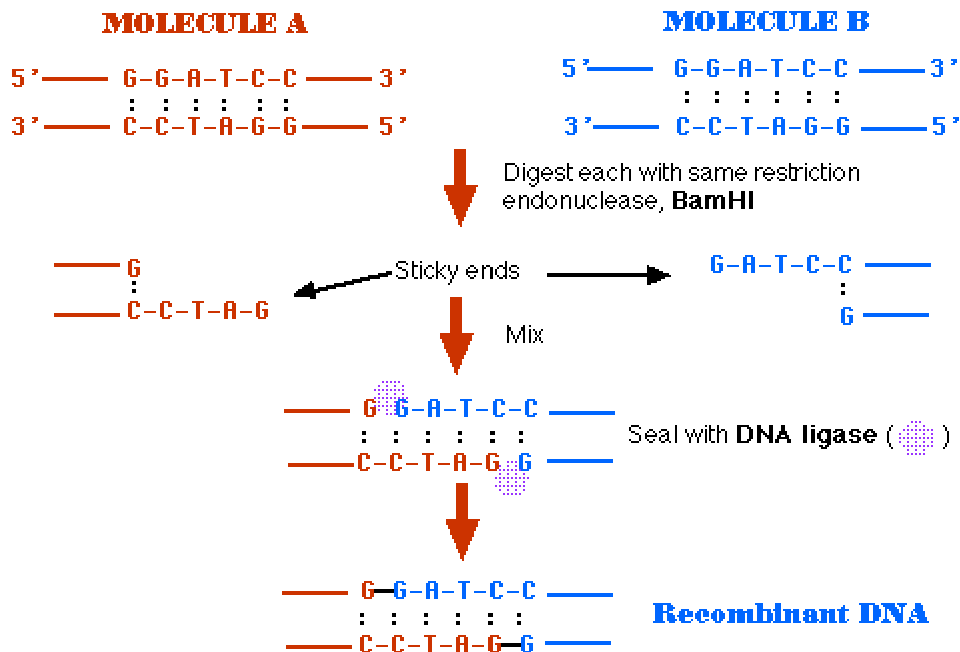
Recombinant DNA and Gene Cloning

Recombinant DNA is DNA that has been created artificially. DNA from two or more sources is incorporated into a single recombinant molecule.

Making Recombinant DNA (rDNA): An Overview



- Treat DNA from both sources with the same restriction endonuclease (BamHI in this case).
- BamHI cuts the same site on both molecules

  5' GGATCC 3'
  3' CCTAGG 5'

- The ends of the cut have an overhanging piece of single-stranded DNA.
- These are called "sticky ends" because they are able to base pair with any DNA molecule containing the complementary sticky end.

- In this case, both DNA preparations have complementary sticky ends and thus can pair with each other when mixed.
- a **DNA ligase** covalently links the two into a molecule of **recombinant DNA**.

To be useful, the recombinant molecule must be replicated many times to provide material for analysis, sequencing, etc. Producing many identical copies of the same recombinant molecule is called **cloning**. Cloning can be done in vitro, by a process called the **polymerase chain reaction** (**PCR**). Here, however, we shall examine how cloning is done **in vivo**.

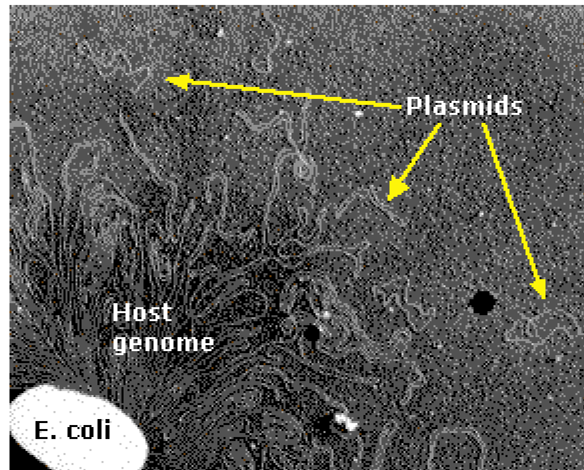
Cloning in vivo can be done in

- unicellular microbes like **E. coli**
- unicellular eukaryotes like **yeast** and
- in mammalian cells grown in tissue culture.

In every case, the recombinant DNA must be taken up by the cell in a form in which it can be replicated and expressed. This is achieved by incorporating the DNA in a **vector**. A number of viruses (both bacterial and of mammalian cells) can serve as vectors. But here let us examine an example of cloning using **E. coli** as the host and a **plasmid** as the vector.

Plasmids

Plasmids are molecules of DNA that are found in bacteria separate from the bacterial chromosome.



Electron micrograph of an E. coli cell ruptured to release its DNA. The tangle is a portion of a single DNA molecule containing over 4.6 million base pairs encoding approximately 4,300 genes. The small circlets are plasmids. (Courtesy of Huntington Potter and David Dressler, Harvard Medical School.)

The desirable properties are:

- are small (a few thousand base pairs)
- usually carry only one or a few genes
- are circular
- have a single **origin of replication**

Plasmids are replicated by the same machinery that replicates the bacterial chromosome. Some plasmids are copied at about the same rate as the chromosome, so a single cell is apt to have only a ´plasmids are copied at a high rate and a single cell may have 50 or more of them.

Genes on plasmids with high numbers of copies are usually expressed at high levels. In nature, these genes often encode proteins (e.g., enzymes) that protect the bacterium from one or more **antibiotics**.

Plasmids enter the bacterial cell with relative ease. This occurs in nature and may account for the rapid spread of antibiotic resistance in hospitals and elsewhere. Plasmids can be deliberately introduced into bacteria in the laboratory transforming the cell with the incoming genes.

PLASMID CLASSIFICATION.

The most useful classification of naturally occurring plasmids is based on the main characteristic coded by the plasmid genes. The 5 main types of plasmids according to this classification are:

1. Fertility plasmids″ F″: Fertility plasmids carry only tra genes(transfer) and have no characteristic beyond the ability to promote conjugal transfer of plasmids.

2. Resistant″ R″ plasmids: They carry genes conferring on the host bacterium resistance to one or more antibacterial agent such as chloramphenicol, ampicillin and mercury. R plasmids are very important in clinical microbiology as they are spread through natural populations and can have profound consequence in the treatment of bacteria infections. Example $RP_{4.}$

3. Col plasmids:They code for colicins. These colicins are proteins that kill other bacteria e.g. colE1 of E.Coli.

4. Degradative plasmids: They allow the host bacterium to metabolise unusual molecules such as Toluene and Salicylic acid e.g. TOL of Plasmodium putida.

5. Virulence plasmids: These confer pathogenicity on the host bacteriume.g. Ti plasmids of agrobacterium tumefaciens, which induce Crown Gall disease on dicotyledonous plants.

An Example:

pAMP

- 4539 base pairs
- a single replication origin

- a gene (**amp**^{r})conferring resistance to the antibiotic **ampicillin** (a relative of penicillin)
- a **single** occurrence of the sequence

  5' GGATCC 3'
  3' CCTAGG 5'

  that, as we saw above, is cut by the restriction enzyme **BamHI**

- a **single** occurrence of the sequence

  5' AAGCTT 3'
  3' TTCGAA 5'

  that is cut by the restriction enzyme **HindIII**

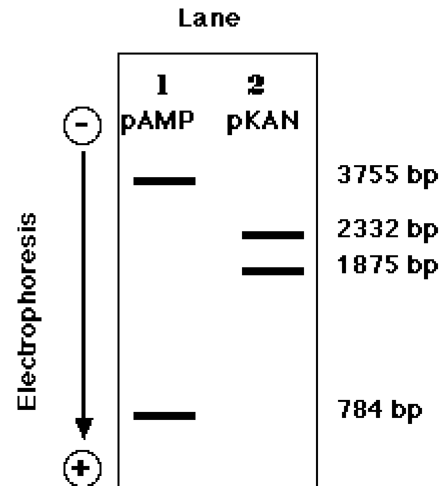
Treatment of pAMP with a **mixture** of BamHI and HindIII produces:

- a fragment of **3755** base pairs carrying both the **amp**^{r} gene and the replication origin
- a fragment of **784** base pairs
- both fragments have sticky ends

pKAN

- 4207 base pairs
- a single replication origin
- a gene (**kan**^{r}) conferring resistance to the antibiotic **kanamycin**.
- a single site cut by **BamHI**
- a single site cut by **HindIII**

Treatment of pKAN with a **mixture** of BamHI and HindIII produces:



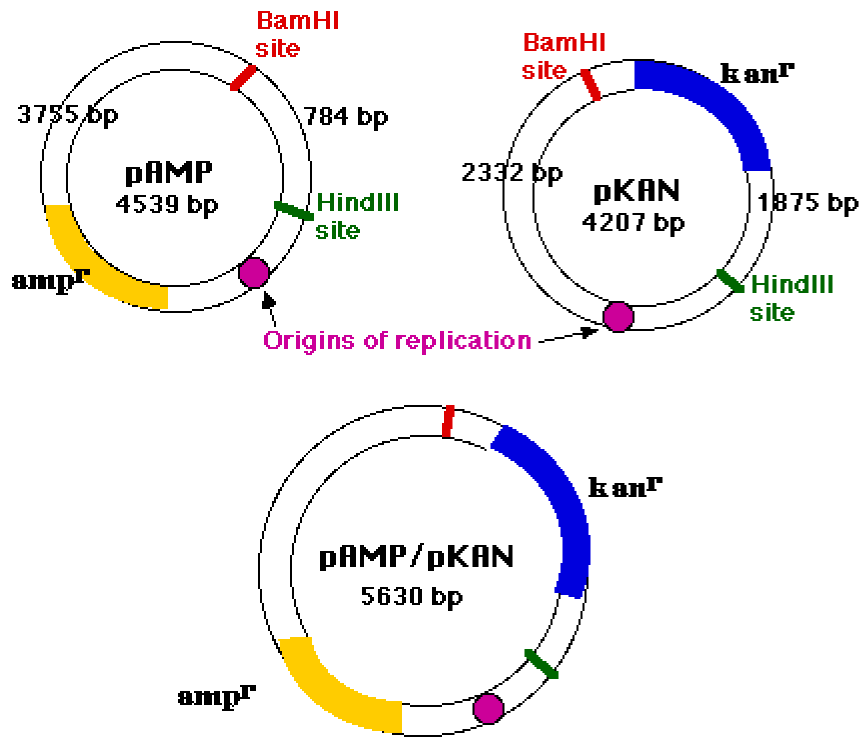
- a fragment of **2332** base pairs
- a fragment of **1875** base pairs with the **kan**^{r} gene (but no origin of replication)
- both fragments have sticky ends

These fragments can be visualized by subjecting the digestion mixtures to electrophoresis in an agarose gel. Because of its negatively-charged phosphate groups, DNA migrates toward the positive electrode (anode) when a direct current is applied. The smaller the fragment, the farther it migrates in the gel.

Ligation Possibilities

If you remove the two restriction enzymes and provide the conditions for DNA ligase to do its work, the pieces of these plasmids can rejoin (thanks to the complementarity of their sticky ends).

Mixing the pKAN and pAMP fragments provides several (at least 10) possibilities of rejoined molecules. Some of these will not produce functional plasmids (molecules with two or with no replication origin cannot function).



One interesting possibility is the joining of

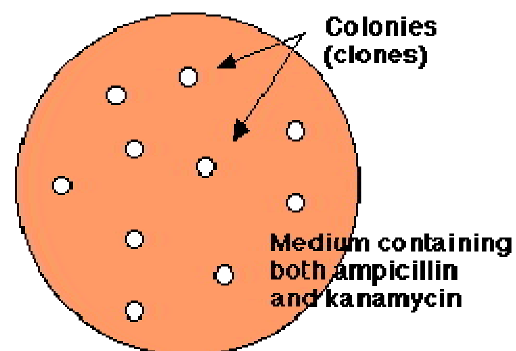
- the 3755-bp pAMP fragment (with **amp**^{r} and a replication origin) with the
- 1875-bp pKAN fragment (with **kan**^{r})

Sealed with **DNA ligase**, these molecules are functioning plasmids that are capable of conferring resistance to **both** ampicillin and kanamycin. They are molecules of **recombinant DNA**.

Because the replication origin, which enables the molecule to function as a plasmid, was contributed by pAMP, pAMP is called the **vector**.

Transforming E. coli

Treatment of E. coli with the mixture of religated molecules will produce some colonies that are able



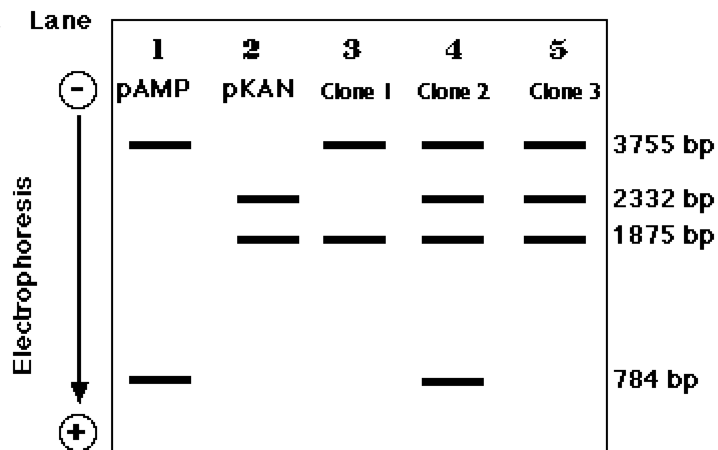
to grow in the presence of both ampicillin and kanamycin.

- A suspension of E. coli is treated with the mixture of religated DNA molecules.
- The suspension is spread on the surface of agar containing both ampicillin and kanamycin.
- The next day, a few cells — resistant to both antibiotics — will have grown into visible colonies containing billions of transformed cells.
- Each colony represents a **clone** of transformed cells.

However, E. coli can be simultaneously transformed by more than one plasmid, so we must demonstrate that the transformed cells have acquired the recombinant plasmid.

Electrophoresis of the DNA from doubly-resistant colonies (clones) tells the story.

- Plasmid DNA from cells that acquired their resistance from a **recombinant plasmid** only show only the **3755**-bp and **1875**-bp bands (**Clone 1**, lane 3).



- **Clone 2** (Lane 4) was simultaneous transformed by religated pAMP and pKAN. (We cannot tell if it took up the recombinant molecule as well.)
- **Clone 3** (Lane 5) was transformed by the recombinant molecule as well as by an intact pKAN.