Chapter 3

Recombinant DNA Technology

Recombinant DNA Technology

- Creation of artificial DNA with two or more sequences that would not normally occur together
- Process of gene splicing
- Involve ligation process
- Plasmids are modified to include a short polylinker sequence
- Contained MCS rich in unique restriction enzyme recognition sequences with DNA ligase

- *HindIII* isolated from *Haemophilus influenzae*
- RE name usually came from genus or species they are isolated from
- Specific sequences bases called recognition sequences or restriction sites
- Cohesive ends: Cut DNA into overhanging strands
- Blund end: Cut DNA with ds, noneoverhanging ends

Restriction Enzyme (RE)

- DNA cutting enzyme with 4-8 nucleotides
- Palindromic which correspond to nitrogenous base sequences that read the same backwards and forwards

  – 5'-GTATAC-3'

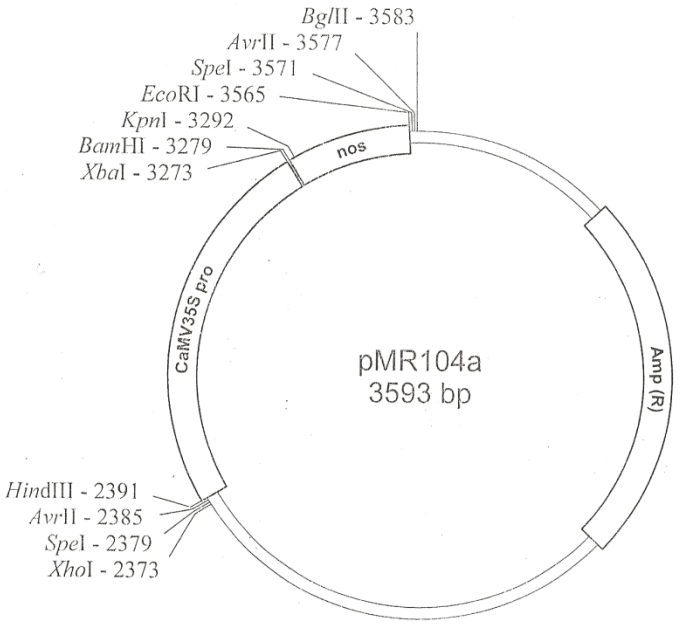
  – 3'-CATATG-5'

Application of RE

- 1. Gene cloning and protein expression
- 2. Distinguish gene alleles
- 3. Analysis of Southern Blot

Plasmid

- Small circular DNA found in bacteria
- Extrachromosomal DNA because they are present in the bacterial cytoplasm in addition to bacterial chromosome
- Size: 1-4kb
- Self replicating



Vector

- A pieces of DNA that can accept, carry and replicate other pieces of DNA
- Characteristics of plasmid:
- 1. Size: Small is easier to manipulate and separated from the chromosomal DNA of host bacteria
- 2. Ori: The site for DNA replication that allow plasmids to replicate from the host cell's chromosome

What makes a good vectors?

- 1. Size: Small is easier to manipulate and separated from the chromosomal DNA of host bacteria
- 2. Ori: The site for DNA replication that allow plasmids to replicate from the host cell's chromosome
- The number of plasmids in the cell called copy number

- 3. MCS/Polylinker: A strech of DNA with recognition sequences for many different common RE (pCAMBIA)
  - Provide great flexibality in the range of DNA fragments to be clone
  - Can choose to cut many RE depend on foreign DNA
- 4. Selectable marker genes
- Allow screening and identification of recombinant plasmid; AmpR, KanR, Lacz

DNA recombinant Selection

- 1. Screening for recombinant plasmid
- Use antibiotics
- Transformed cells will survived in media contained antibiotics
- Nontransformed: do not grow

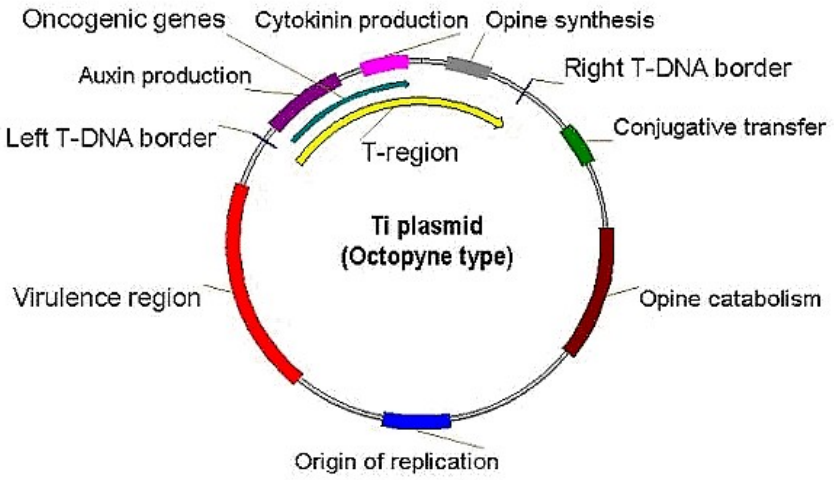
2. Blue white screening

- The competent cells are grown in the presence of X-gal
- Recombinnat: white whilw nonrecombinant is blue
- Less time and less labor intensive

- IPTG; isopropyl β-D-1 thiogalactopyranoside, inducer of the Lac operon
- The hydrolysis of colourless, X-gal by the β-galactosidase causes the characteristic blue colour in the colonies
- Its shows that the colonies contain vector without insert (non-transformed)

Type of vectors

- Researchers worked out to develop vector with particular benefits
- Ref Table 3.2 pp 66
  - Bacterial plasmid vector (circular and linear)
  - Cosmid
  - BAC
  - YAC
  - Ti



Collection of Cloned Genes

- Begin with preparing DNA library

- Typically for cloning:
  - Genomic cDNA library
  - Complementary DNA libraries (cDNA)

Genomic Library

- Chromosomal DNA from the tissue of interest is isolated and digested with RE
- Organism's entire genome
- Use DNA ligase to ligate genomic DNA pieces and vector DNA randomly
- Disadvantage:
  - Nonprotein coding pieces of DNA called introns are cloned
  - Many clones contained nonprotein coding DNA
  - Many have large genome: difficult

cDNA libraries

- mRNA were used to making library
- However, mRNA cannot be cut with RE. So they must be replicated to cDNA. This will achieved by RT-PCR
- Conversion of RNA to cDNA (complemetary DNA: exact copy of mRNA)
- Involved Reverse Transcriptase enzyme

Advantages of cDNA libraries

- Collection of actively expressed genes
- Introns are not cloned
- Preferred compared to genomic library
- They can be created and screened to isolated genes that are primarily expressed in certain tissue