Chapter 8

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Recombinant DNA technology

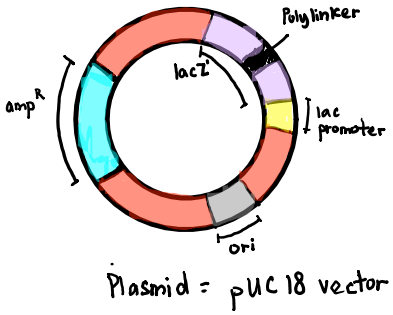
Definition: A technology to produce recombinant DNA molecule.

Recombinant DNA molecule

Definition:
Fragments of DNA from 2 different species , spliced together in the laboratory (in vitro) into a single molecule.

Tools in recombinant DNA technology

1. Target DNA (with gene of interest)
   - Gene or DNA fragments to be cloned
2. DNA cloning vector (Plasmid)
   - DNA molecule carry foreign DNA fragment into a host cell and replicated there.

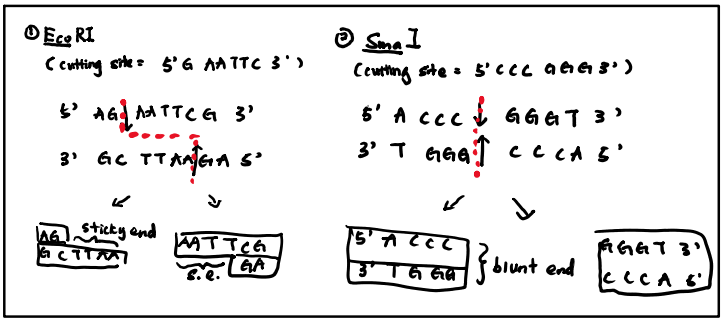


Plasmid = pUC 18 vector

Characteristic of plasmid

1. Able accept foreign DNA in MCS (Multiple Cloning Sites)
2. Able replicate freely in host cell (because has origin of replication, ori)
3. Has selectable marker gene (amp R - resist to antibiotic)
4. Able express/ amplify cloned gene.

3. Restriction enzyme (Endonuclease)
   - Enzyme that recognise and cut specific base sequence of DNA at restriction site.



Characteristic of host cell

1. Able receive recombinant dna through transformation
2. Able maintain structure of recombinant dna
3. Able to amplify gene product from recombinant dna
4. Able express/ amplify gene of interest

4. Modifying enzyme
   - DNA ligase
   - Enzyme join gene of interest and cloning vector by forming phosphodiester bonds
   - Taq polymerase
   - DNA polymerase used for dna amplification which tolerate to high temperature (>60°C)

5. Host cell (E.coli)
   - Organism receives recombinant dna for cloning purpose.

Gene cloning

1. Isolation of dna
   - Isolate target DNA from donor cell
   - Isolate plasmid from bacterial cell

2. Cut
   - Cut both target dna and plasmid with same restriction enzyme to make complementary sticky/ blunt end
   - Restriction enzyme cut target dna at mcs (multiple cloning site)
   - Restriction enzyme cut plasmid at restriction site in lac Z

3. Insertion
   - Gene of interest inserted into open plasmid
   - Gene of interest and plasmid are join by **dna ligase**
   - Recombinant dna formed

4. Transformation and amplification
   - Recombinant dna introduced into host cell through **transformation**
   - Recombinant dna amplified in host cell
   - By forming colonies of host cell.

5. Screening
   - Blue-white screening for positive clone
   - By plate bacteria on medium containing ampicillin and X-gal

Bacteria with recombinant plasmid had non-functional lac Z
Non-functional lac Z cannot produce b-galactosidase to

By forming colonies of host cell.

5. Screening
- Blue-white screening for positive clone
- By plate bacteria on medium containing ampicillin and X-gal
- Bacteria with recombinant plasmid form white colonies
- Bacteria without recombinant plasmid form blue colonies.

Bacteria with recombinant plasmid had non-functional lac Z
Non-functional lac Z cannot produce b-galactosidase to hydrolyse X-gal. form whiteZ

Bacteria with recombinant plasmid has functional lac Z produce b-galactosidase to hydrolyse X-gal, form blue

PCR (Polymerase chain reaction)

1. Denaturation
- Mixture with dna strands heated to high temperature (94-98°C) to denature double stranded dna
- Make dna fragment become single stranded.
- Both strands of dna act as template.

2. Annealing
- Temperature gradually cooled (50-60°C)
- Single-stranded dna primer anneal complementary to sequences on the opposite strands at each end of target sequence
- One primer is complementary to one end of target sequence of one strand
- Second primer is complementary to other end of sequence of other strand

3. Extension
- Temperature increase to (70-72°C)
- **Taq polymeras**e catalyse addition of and nucleotides in 5' to 3' direction.
- Complementary dna strand produced.

RT-PCR (Reverse transcription polymerase chain reaction)

1. mRNA isolated from a sample of cells into test tube
2. **Reverse transcriptase** catalyse synthesis of first single cDNA strand using mRNA as template
3. mRNA degrades by another enzyme
4. DNA polymerase catalyses synthesis of second DNA strand
5. Double stranded cDNA formed
6. It amplify by PCR

Production of human insulin

1. Isolate mRNA from human pancreatic cell
2. mRNA used as template for reverse transcription
3. Catalyse by reverse transcriptase
4. Produce cDNA
5. DNA polymerase added to synthesis second strand
6. Double stranded cDNA form
7. cDNA and plasmid are cut with same restriction enzyme
8. To produce complementary sticky end
9. cDNA inserted into open plasmid
10. Joined by DNA ligase
11. Recombinant DNA formed.
12. Recombinant Dna introduce into host cell by transformation
13. Recombinant dna amplified in host cell
14. Screening of recombinant bacteria by blue-white screening

Definition:

1. recombinant dna technology: a technology used to produce recombinant dna molecule
2. Recombinant dna molecule: fragments of dna from two species spliced together in vitro into a single molecule (exp: bacterium and mammal)
3. Target dna : dna fragment contain gene of interest and be cloned
4. Dna cloning vector: dna molecule that can carry foreign dna fragment into a host cell and replicated there
5. Restriction enzyme: enzyme that recognise and cut specific base sequence of dna at restriction site
6. Dna ligase: enzyme join gene of interest and cloning vector by forming phosphodiester bond
7. Taq polymerase : a type of dna polymerase that used for dna amplification in pcr withstand to high temp.
8. Host cell : organisms receives recombinant dna for cloning purpose
9. PCR: a method of cloning dna fragment in vitro
10. RT-PCR: a technique combining reverse transcription of RNA into cDNA and amplification of cDNA using PCRe