KMPk RECOMBINANT DNA TECHNOLOGY DB014

BIOLOGY WORKSHEET

CHAPTER 7: RECOMBINANT DNA TECHNOLOGY

Subtopic : 7.1 Introduction To Recombinant DNA Technology

Learning Outcome :

i. **Define recombinant DNA technology**
ii. **List the tools used in recombinant DNA technology and state their function:**

- ✓ **Target DNA (gene of interest)**
- ✓ **Restriction enzyme**
- ✓ **DNA cloning vector**
- ✓ **Host cell**
- ✓ **Modifying enzymes (DNA ligase)**

PART A: MULTIPLE CHOICE QUESTIONS

1) The enzymes below are used in genetic engineering except

A. Reverse transcriptase
B. Restriction enzymes
C. Replicase
D. DNA polymerase

2) The function of restriction enzymes is to

A. Join nucleotides during replication
B. Join nucleotides during transcription
C. Add new nucleotides to the growing strand of DNA
D. Cut nucleic acids at specific area.

3) The term 'sticky ends' refers to

A. Double–stranded ends of a DNA segment
B. Loops of single-stranded DNA
C. Single-stranded ends of a DNA segment that pair with complementary single-stranded ends.
D. Sites of origin of replication in prokaryotes

4) Genes from one organism can be transferred to another using a

A. Vector
B. Reverse transcriptase
C. Genetic probe
D. PCR method

5) Plasmids are used in recombinant DNA technology as

A. Recognition sites on recombinant DNA strands
B. Surfaces for respiratory processes in bacteria
C. Surfaces for protein synthesis in bacteria
D. A vehicle for the insertion of recombinant DNA into bacteria

6) Which of the following can be used as cloning vector?

I. Lambda phage

II. *Eco R1*

III. Bacterial plasmid

IV. *E. coli*

A. I, II and III

B. 1 and III

C. II and IV

D. I and IV

7) Which of the following statements about plasmid is not true?

A. A part of bacterial chromosome

B. Found in bacterial cell

C. Able replicate freely

D. A small circular DNA

8) A large number of copies of any DNA segment can be obtained by:

A. Introducing foreign DNA into a microorganism so that it can be replicated.
B. Stimulating increased transcription of the appropriate sequence of mRNA.
C. Stimulating increased translation of the appropriate DNA molecule.
D. Stimulating the production of DNA from proteins.

9) Bacteriophages are:

A. Viruses that infected bacteria
B. Plasmids that infect bacteria
C. Bacteria that infect other bacteria
D. Enzymes that destroy bacteria

10) What purpose do restriction enzymes play in bacterial cells?

A. Restriction enzymes prevent the overproduction of mRNA in the bacterial cell.
B. Restriction enzymes attack bacteriophage DNA when it enters the cell.
C. Restriction enzymes promote bonding of the promoter to the mRNA molecule
D. Restriction enzymes limit the rate of bacteria replication.

11) Transformation is a process whereby:

A. Bacteria are transferred into plasmid cells
B. Viruses are transferred into bacterial cells
C. Plasmids are transferred into bacterial cells
D. Bacterial are transferred into viral cells

PART B: STRUCTURED QUESTIONS

1. Formation of Recombinant DNA

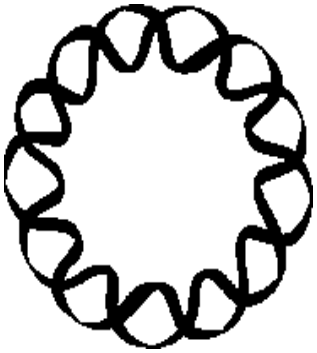
I. Cut out both plasmid and targeted DNA below.

II. By using a pair of scissors (**restriction enzyme**), cut the plasmid at the palindromic site.

III. Insert the targeted DNA at the palindromic site.

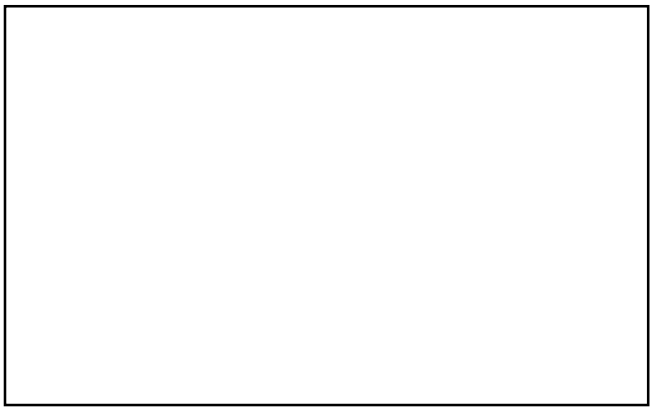
IV. Use glue (**modifying enzyme**) to stick the targeted DNA to the plasmid.

V. Stick the recombinant DNA in the space provided.



Plasmid

Targeted DNA



Definition of Recombinant DNA:

2. Below are the terms of tools used in the recombinant DNA technology. Visualize the tools and write the definition for the tools.

Target DNA

Definition

Restriction enzyme

Definition

DNA cloning vector

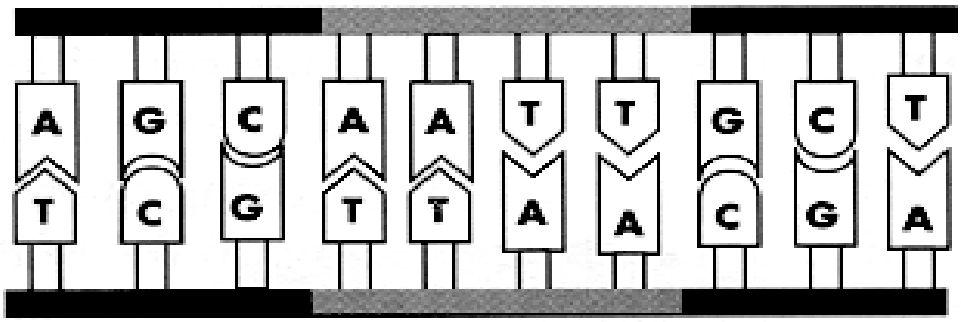
Definition

Host cell

Definition

Modifying enzyme
Definition

3. By using *Eco*RI, cut the targeted DNA at the palindromic site to produce a sticky end



Targeted DNA

4. Restriction enzymes are widely used in genetic engineering. FIGURE 1 shows the act of restriction enzyme on DNA.

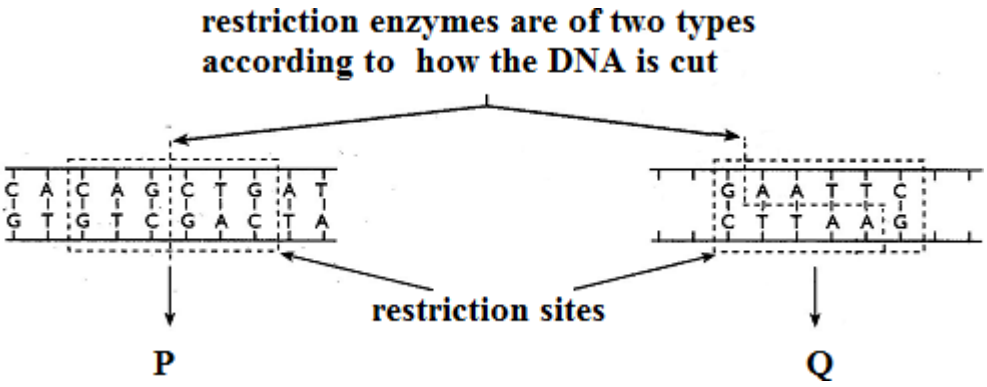


FIGURE 1

a) What is meant by restriction enzyme?

_____

[1 mark]

b) Based on how the DNA is cut, structure **P** and **Q** will be formed.

i. Name **P** and **Q**.

P: _____

Q: _____

[2 marks]

ii. Draw structures **P** and **Q** in the space given below.

[2 marks]

iii. State the term for the base sequences in the DNA that is cut by restriction enzymes.

_____

[1 mark]

iv. Name the restriction enzyme that produces fragment **Q**.

_____

[1 mark]

c) List the important characteristics of plasmid vectors used in recombinant DNA technology.

_____

_____

[3 marks] _____

5. FIGURE 2 shows the base sequence in parts of a foreign DNA and the cut plasmid showing sticky ends.

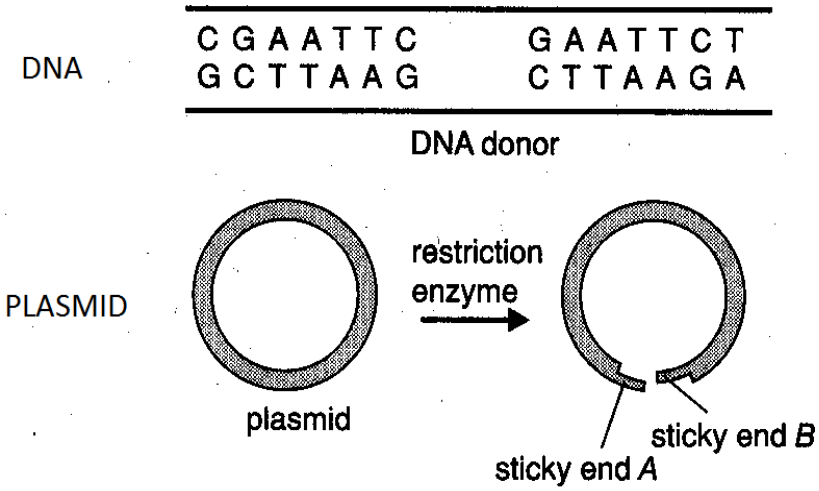


FIGURE 2

a) What is the function of the plasmid shown?

_____

[1 mark]

b) Identify the palindromic base sequence in the DNA donor molecule shown.

_____

[1 mark]

c) Name the restriction enzyme used to cut both the foreign DNA molecule and plasmid.

_____

[1 mark]

d) A restriction enzyme will cut the DNA donor molecule and plasmid to produce restriction fragments. Draw the restriction fragments of the foreign DNA molecule produced.

[3 marks]

e) Draw the recombinant DNA formed as the result of inserting the DNA restriction fragments into the cut bacterial plasmids.

[2 marks]

f) Name the enzyme that used to seal both DNA and plasmid.

_____

[1 mark]

g) What is the next stage after formation of recombinant DNA?

_____

[1 mark]

6. Explain the characteristics of plasmid as cloning and expression vector (C2).

7. Explain the characteristic of *E.coli* as host cell (bacteria) and its characteristics (C2).

Tool	Characteristic
Plasmid	
E.coli	

8. Explain modifying enzyme and its function (C2):

Modifying enzyme	Function
DNA ligase	
***Taq* polymerase**	

BIOLOGY WORKSHEET

CHAPTER 7 : RECOMBINANT DNA TECHNOLOGY

Subtopic : 7.2 Methods In Gene Cloning

Learning Outcome :

a) Overview using diagram to show the steps in gene cloning by using plasmid.

b) Describe the steps in gene cloning by using plasmid as the vector:

- **i. Isolation of gene**
- **ii. Cleave / cut**
- **iii. Insertion**
- **iv. Transformation and amplification**
- **v. Screening (blue / white screening)**

PART A: MULTIPLE CHOICE QUESTIONS

1. In order to insert a foreign gene into a plasmid, both must _____

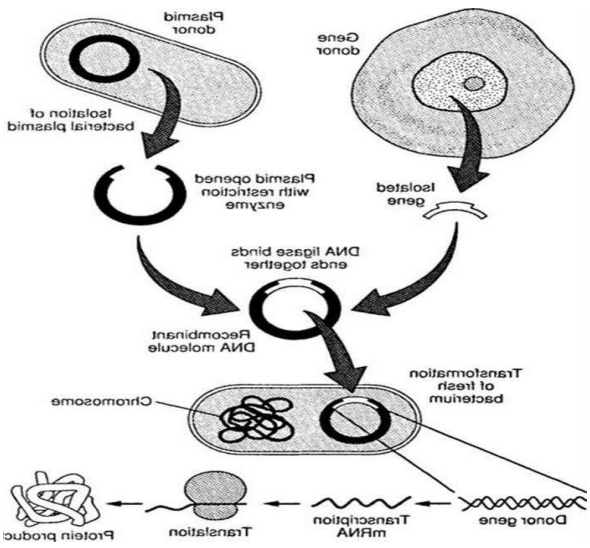
   A. have identical DNA sequences

   B. originate from the same type of cell

   C. be cut by the same restriction enzyme

   D. be of the same length

2. A recombinant DNA molecule is produced by



   A. Joining of two DNA fragments
   B. Joining of two or more DNA fragments
   C. Both A and B
   D. Joining of two or more DNA fragments originating from different organisms

3. The gene formed by the joining of DNA segments from two different sources are called as

   A. Recombinant gene
   B. Joined gene
   C. Both A and B
   D. Chimaeric gene

4. Which of the following enzyme is used to cut DNA molecule in rDNA technology?

   A. Ligase
   B. Phosphate
   C. Ribonuclease
   D. Restriction enzyme

5. Restriction enzymes are called as

   A. Biological scissors
   B. Molecular scalpels
   C. Molecular knives
   D. All of these

6. All of theseThe DNA molecule to which the gene of insert is integrated for cloning is called
   A. Carrier
   B. Transformer
   C. Vector
   D. None of these

7. The DNA segment to be cloned is called
   A. Gene segment
   B. DNA fragment
   C. DNA insert
   D. All of these

8. The mechanism of intake of DNA fragments from the surrounding medium by a cell is called
   A. Transformation
   B. Amplification
   C. Both A and B
   D. Conjugation

9. What is the purpose of amplification?

A. To screen for recombinant bacteria.
B. To join the rDNA with the host cell.
C. To produce multiple copies of the desired gene
D. To prevent the bacterial from reproducing

10. The following steps involved in gene cloning procedures. Rearrange them into the CORRECT sequences:

I. Transformation and amplification.
II. Screen for bacteria that contain recombinant DNA.
III. Insert DNA fragment into the cut bacterial plasmid.
IV. Cleave the plasmid and human DNA with the same restriction enzyme.
V. Isolate the bacterial plasmid and human DNA.

A. I, II, III, IV, V
B. V, IV, III, I, II
C. IV, V, III, II, I
D. V, IV, II, III, I

11. All fragments cut by most restriction endonucleases have

A. complementary double-stranded ends
B. supplementary single-stranded ends
C. double-stranded "sticky" ends
D. complementary single-stranded ends

12. Which of the following is TRUE regarding blue white screening?

I. Antibiotics will prevent the growth of bacteria without plasmid.
II. Bacteria cells with recombinant plasmids are resistant to antibiotics and are unable to hydrolyse X-gal.
III. Bacteria cells which contain non-recombinant plasmids are resistant to antibiotics and are unable to hydrolyse X-gal.

A. I only
B. I and II only
C. II and III only
D. All of the above

13. The second step in most genetic engineering experiments is

A. Screening
B. production of recombinant DNA
C. cleavage of DNA
D. cloning

14. In the screening process, clones that metabolize X-gal turn

A. White
B. Orange
C. Red
D. Blue

15. One of the most useful methods for identifying a specific gene is

A. screening
B. blotting
C. chromatography
D. magnetic resonance imaging

16. Which of the following is the CORRECT sequence in the cloning process?

I. splice the DNA fragment into the vector
II. cut DNA of the vector
III. cut the source DNA
IV. transform the vector into the host

A. I, II, IV, III
B. II, I, III, IV
C. II, III, I, IV
D. II, IV, I, III

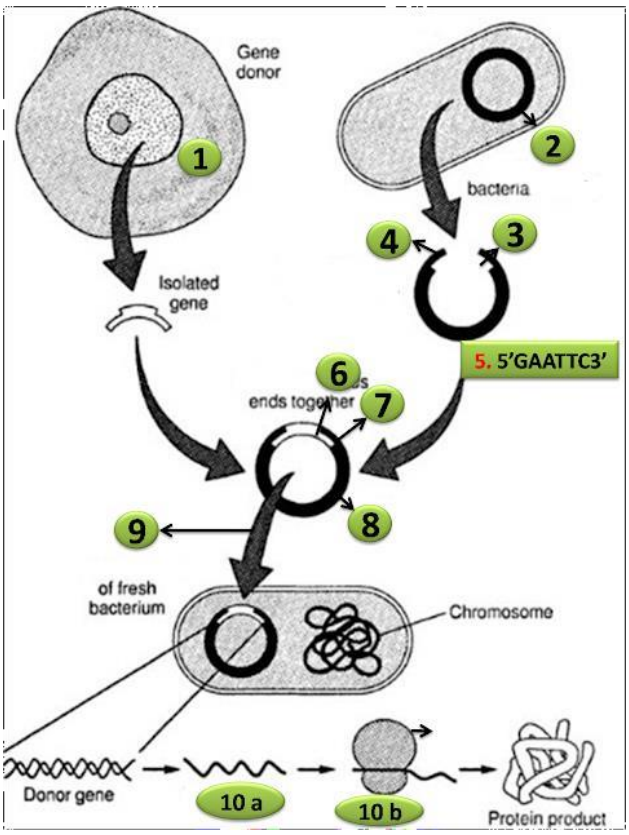
17. Identification of bacteria containing recombinant plasmids can be done by

A. Using radioactive probes to locate the plasmids.
B. Using culture media containing an antibiotics that kills the cells without the recombinant plasmid.
C. Removing the DNA of all cells in a culture to see which cells have the recombinant plasmid.
D. Producing antibodies specific for each bacterium containing a recombinant plasmid.

18. To make the recombinant plasmid permeable to DNA molecules, which of the chemicals is added?

A. $MgCl_2$
B. $CaCl_2$
C. NaCl
D. HCl

PART B : STRUCTURED QUESTIONS



The first step in rDNA technology, labelled 1 is _____2. It is double stranded, self-replicating, circular DNA molecule present in bacteria which is widely used as a gene cloning vector. The structure labelled 2 in the figure is _____3. These enzymes are called as molecular scissors which is essential for making internal cuts in a DNA molecule or vector at specific sites. The enzyme used in making cut in the vector (labelled 3) is _____4. The cut by the enzyme in the vector (labelled 4) creates single stranded unpaired regions of DNA. This type of cut pattern is called as _____ 5. 5'GAATTC3' is a restriction site of a widely used restriction enzyme which produces sticky ends. The enzyme is _____6. In the figure labelled 6, the DNA strand is the _____7. This enzyme is called as molecular glue which is used to join two DNA strands by forming phosphodiester bond. The joining enzyme labelled 7 is _____8. The vector (plasmid) with foreign gene inserted is called (labelled 8) _____9. The figure labelled 9 is the process of introducing recombinant vector into a suitable host like bacterium. The process is called _____10. In the figure, 10 a and 10 b are processes that lead to the formation of protein product encoded by the gene of interest. 10 a and 10 b are _____.

2. **FIGURE 2** shows an outline of the processes involved in the production of useful genes and proteins by genetic engineering.

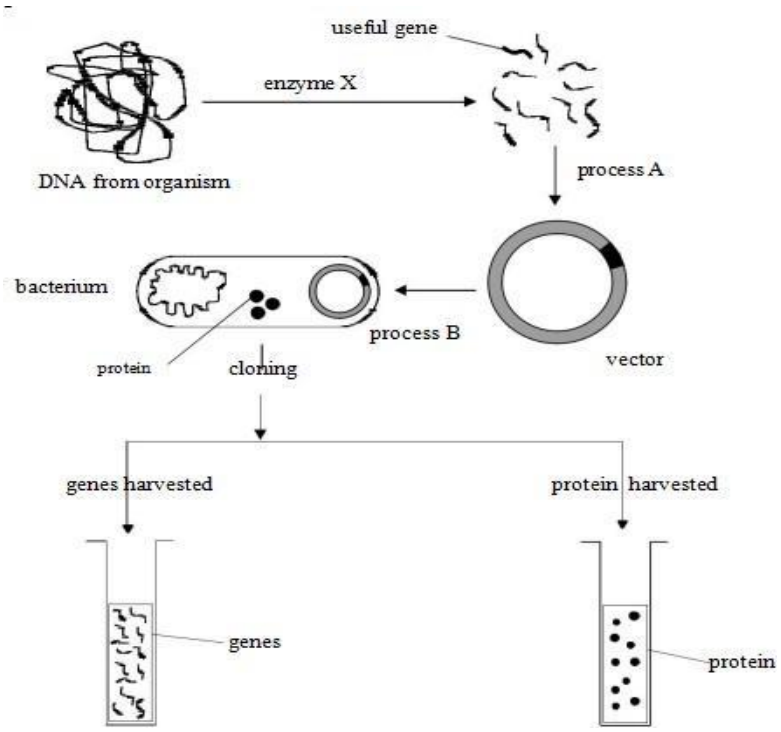


FIGURE 2

(a) (i) Name enzyme X.

[1 *mark*]

(ii) Name the enzyme which will be used to open the vector.
[1 *mark*]

(b) What is the likely nature of the vector? [1 *mark*]

(c) Outline process **B.** [3 *marks*]

(d) Outline the process which takes place inside the bacterium. [2 *marks*]