جامعة بنـها الكلية معتمدة بالقرار (154) بتاريخ 2016/5/23 كلية الزراعة

قسم الوراثة والهندسة الوراثية الفصل الدراسي الأول (2020/2019)

برنامج التكنولوجيا الحيوية الزراعية الفرقة الثالثة الزمن ساعتين

البيولوجيا الجزيئية **Molecular Biology**

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ANSWER THE FOLLOWING QUESTIONS:

First question: (20 Marks)

Write Notes about :

(1): Lac operon and Trp operon in bacteria .

(2): In Gene Cloning Method:

Why clone DNA –Sources of DNA for cloning –Cloning tools – Plasmid and Cosmid as a cloning vectors- What determines the choice vectors - and How to Clone DNA ?

Second Question : (20 Marks)

Explain briefly only two points

(1): The main steps from gene to protein.

(2): Types of DNA damage and type of photoreactivation repair and Mismatch repair.

(3) Difference between Structural and Regulatory Genes.

Third Question : (20 Marks):

(1): *Explain*:

The basic procedures of recombinant DNA technology and its applications**.**

(2): Complete The following (Only five Points)

(1): The function of ribosomes are ---------- and ----------

(2): Transcription factors act to ----------

(3): Excision repair accomplished by many enzymes----------

(4):---------- a section of bacterial DNA that regulates the transcription of structural genes in an operon.

(5):---------- is a functioning unit of DNA containing a cluster of genes under the control of a single promoter.

(6): A facultative gene is a ----------

(7): Type of operon are ---------- and ----------

With my best wishes

Prof. Dr/ Mohamed Serag Eldin

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الفصل الدراسي الأول (2020/2019)

Molecular Biology

Model Answer

First question : (20 Marks)

(1): Lac operon and Trp operon in bacteria .

The lactose operon (Lac operon)

The lactose operon contains 3 genes, Lac Z, Y and A. These genes encode for enzymes required to metabolize lactose --> beta-galactosidase, lactose permease, and beta-galactoside transacetylase.

Another regulatory gene, Lac I, is expressed separately and lies upstream of the operon. This gene codes for the lac repressor which regulates the expression of Lac Z, Y and A

Control Circuit for the lac Operon

I P O || Z | Y | A |

_____

Controlling || Structural genes
Region

***In the absence of lactose**:*

The lac repressor bind to a DNA sequence called the "operator" (found between the lac Z gene and the lac promoter)

In this way the lac repressor blocks the path of RNA polymerase to reach the lac Z,Y and A genes --> operon remains switched off.

In the presence of lactose:

Lactose molecules are metabolized by the lac enzymes, an intermediate is formed called all lactose (an isomer of lactose)

All lactose acts as an inducer by binding to the lactose repressor and changing its conformation --> therefore it can no longer bind to the operator.

The regulatory protein "CAP" (Catabolite Activator Protein) binds to a DNA sequence upstream to the lac promoter and enhances binding of the RNA polymerase leading to an increased transcription of the operon.

The lac Operon - an inducible system

Lactose ----------> Glucose + Galactose

ß-galactosidase

Several proteins involved in lactose metabolism in the E. coli cell. They are:

ß-galactosidase - converts lactose into glucose and galactose

ß-galactoside permease - transports lactose into the cell

ß-galactoside transacetylase - function unknown

Operon - a cluster of structural genes that are expressed as a group and their associated promoter and operator

<u>The Tryptophan operon (Trp operon)</u>

It includes 5 genes involved in Tryptophan synthesis

The genes are expressed as a single mRNA strand, transcribed from an upstream promoter

Another regulatory gene encodes for a trp repressor.

There is also the trp operator that is found just downstream from the promoter

I**f tryptophan is present** it will bind the trp repressor. This enables it to bind the operator and block the RNA polymerase. This is a model of end-product inhibition, since tryptophan is the end product of tryptophan biosynthesis.

The trp mRNA can form 2 stem-looped structures between the trp-promoter and the 1st trp gene. The large stem loop does not influence transcription and occurs upstream of the smaller stem loop which acts as a transcription terminator. If the large stem loop is not present then the short stem-loop will be formed.

The formation of the stem-loops on the mRNA depends on the binding of ribosomes to the mRNA.

Just prior to the stem-loop region fo the mRNA, there is an open reading frame of 14 codons, of which 2 of them code for tryptophan.

*If **the tryptophan is present** in adequate levels in the cytoplasm, it will be translated and the ribosome will keep translocating following closely behind the RNA polymerase preventing the formation of the large loop structure, allowing the terminator structure to form further on and transcription ends.*

***If tryptophan is lacking**, the ribosome will be stalled as it tries to translate the coding region. This will allow the RNA polymerase to move ahead and leave enough space for the larger stem-loop structure to form, thus allowing the transcription to continue.*

(2): In Gene Cloning Method:

Why Clone DNA : A particular gene can be isolated and its nucleotide sequence determined

Control sequences of DNA can be identified & analyzed ▪

Protein/enzyme/RNA function can be investigated ▪

Mutations can be identified, e.g. gene defects related to specific diseases. ▪

Organisms can be 'engineered' for specific purposes, e.g. insulin production, insect resistance, etc. ▪

Sources of DNA for cloning :

Chromosomal DNA and RNA converted to cDNA

Cloning Tools

Restriction endonucleases ,Ligase ,Vectors ▪

Methods for introducing DNA into a host cell. ▪

Plasmid vectors

may encode genetic information for properties ▪

1 Resistance to Antibiotics, 2 Bacteriocins production ▪

3 Enterotoxin production , 4 Enhanced pathogen city ▪

5 Reduced Sensitivity to mutagens, ▪

6 Degrade complex organic molecules. ▪

Plasmid vectors

Plasmid vectors are double-stranded, circular, self-replicating, extra-chromosomal DNA molecules.

Advantages:

Small, easy to handle

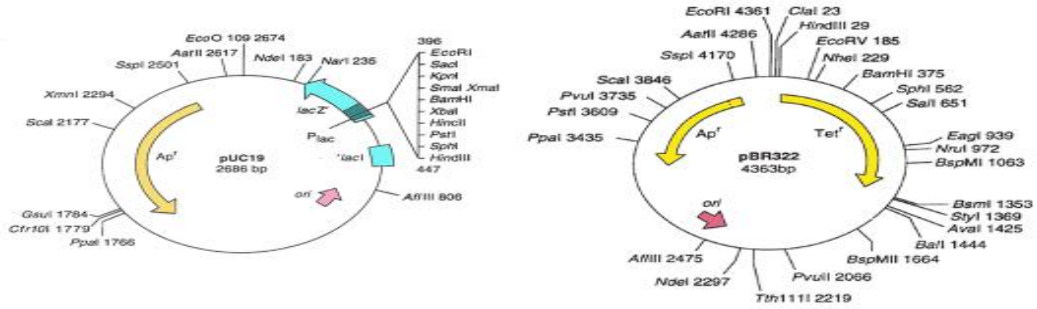
Straightforward selection strategies

Useful for cloning small DNA fragments (< 10kbp)

Disadvantages:

Less useful for cloning large DNA fragments (> 10kbp)

Plasmid vectors



Cosmid vectors

Combine the properties of plasmid vectors with the useful properties of the l cos site

Advantages:

Useful for cloning very large DNA fragments (32 - 47 kbp) ▪

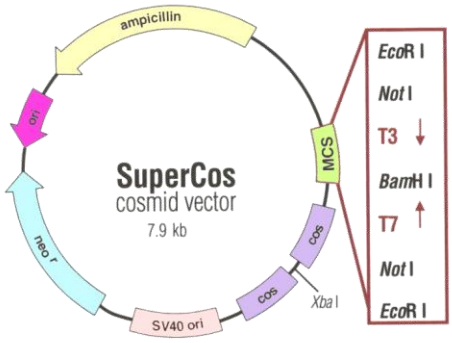
Inherent size selection for large inserts ▪

Handle like plasmids ▪

Disadvantages: ▪

Not easy to handle very large plasmids ▪

(~ 50 kbp) ▪

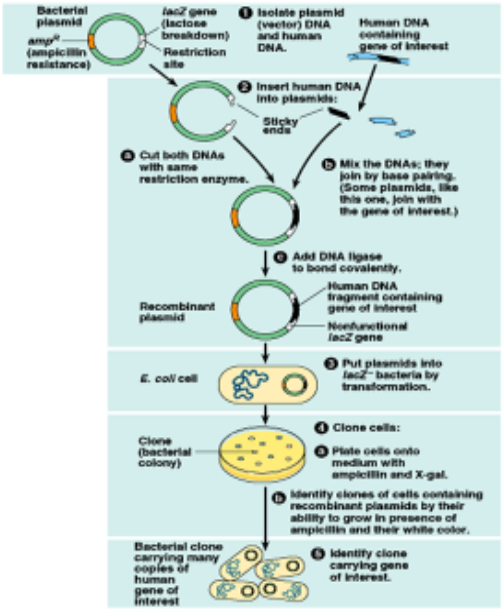


What determines the choice vector? ▪

Insert size – Vector size – Restriction sites – Copy number- ▪
Cloning efficiency – Ability to screen for inserts.

How to clone DNA : ▪

- Isolation of cloning vector (bacterial plasmid) & gene-source DNA (gene of interest)
- Insertion of gene-source DNA into the cloning vector using the same restriction enzyme; bind the fragmented DNA with DNA ligase
- Introduction of cloning vector into cells (transformation by bacterial cells)
- Cloning of cells (and foreign genes)
- Identification of cell clones carrying the gene of interest



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Second Question : (20 Marks)

Explain briefly only two points

Protein synthesis by Transduction and translation.

Transcription proceeds through:

Initiation – RNA polymerase identifies where to begin transcription.
Elongation – RNA nucleotides are added to the 3’ end of the new RNA.
Termination – RNA polymerase stops transcription when it encounters terminators in the DNA sequence.

Translation proceeds through:
Initiation – mRNA, tRNA, and ribosome come together.

Elongation – tRNAs bring amino acids to the ribosome for incorporation into the polypeptide.
Termination – ribosome encounters a stop codon and releases polypeptide.
(1): The main steps from gene to protein.

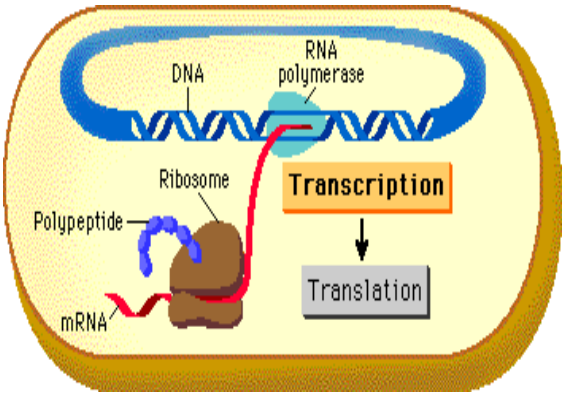
Concept 1: The Central Dogma

The central dogma of molecular biology describes the two-step process, transcription and translation, by which the information in genes flows into proteins: DNA → RNA → protein.
Transcription is the synthesis of an RNA copy of a segment of DNA. RNA is synthesized

Concept 2: Transcription and Translation in Cells

In a prokaryotic cell, transcription and translation are coupled; that is, translation begins while the mRNA is still being synthesized. In a eukaryotic cell, transcription occurs in the nucleus, and translation occurs in the cytoplasm.

Prokaryotic Cell



Because there is no nucleus to separate the processes of transcription and translation, when bacterial genes are transcribed, their transcripts can immediately be translated.

Eukaryotic Cell

Transcription and translation are spatially and temporally separated in eukaryotic cells; that is, transcription occurs in the nucleus to produce a pre-mRNA molecule.

The pre-mRNA is typically processed to produce the mature mRNA, which exits the nucleus and is translated in the cytoplasm.

Concept 3: Different Genes for Different RNAs

There are 4 types of RNA, each encoded by its own type of gene.

The genomic DNA contains all the information for the structure and function of an organism.

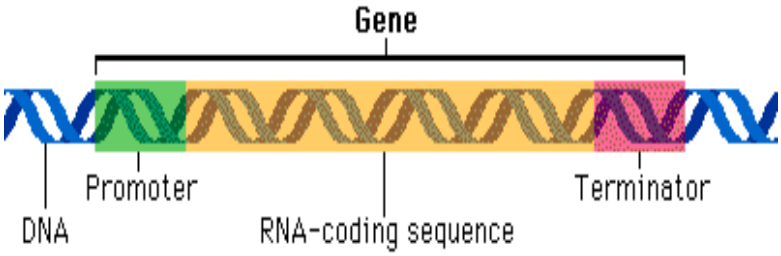
In any cell, only some of the genes are expressed, that is, transcribed into RNA.

There are 4 types of RNA, each encoded by its own type of gene:

- mRNA - Messenger RNA: Encodes amino acid sequence of a polypeptide.
- tRNA - Transfer RNA: Brings amino acids to ribosomes during translation.
- rRNA - Ribosomal RNA: With ribosomal proteins, makes up the ribosomes, the organelles that translate the mRNA.
- snRNA - Small nuclear RNA: With proteins, forms complexes that are used in RNA processing in eukaryotes. (Not found in prokaryotes.)

Concept 4: Basic Structure of a Protein-Coding Gene

A protein-coding gene consists of a promoter followed by the coding sequence for the protein and then a terminator.



The promoter is a base-pair sequence that specifies where transcription begins.

The coding sequence is a base-pair sequence that includes coding information for the polypeptide chain specified by the gene.

The terminator is a sequence that specifies the end of the mRNA transcript.

Concept 5: The RNA Molecule

RNA is structurally similar to DNA.

Concept 6: The Transcription Process

RNA synthesis involves separation of the DNA strands and synthesis of RNA molecule in the 5' to 3' direction by RNA polymerase, using one of the DNA strands as a template.

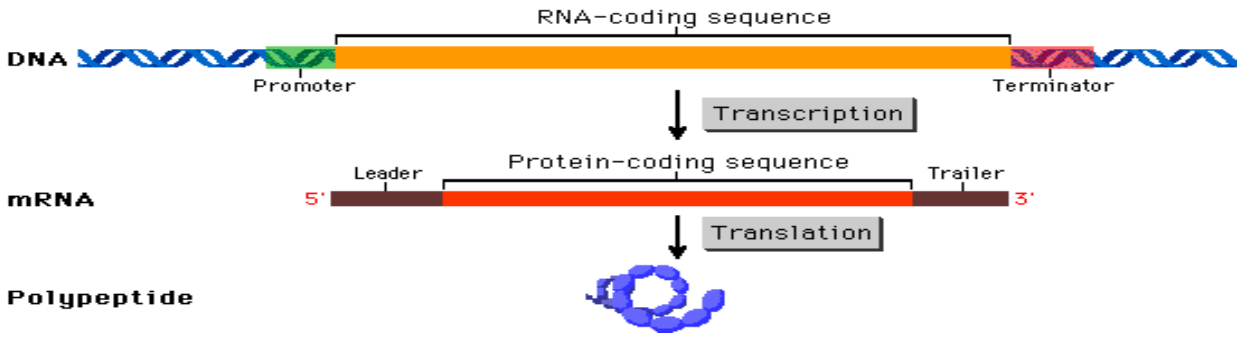
In complementary base pairing, A, T, G, and C on the template DNA strand specify U, A, C, and G, respectively, on the RNA strand being synthesized.

Concept 7: Complete Transcription of an RNA Molecule

Transcription begins at the promoter, proceeds through the coding region, and ends at the terminator.

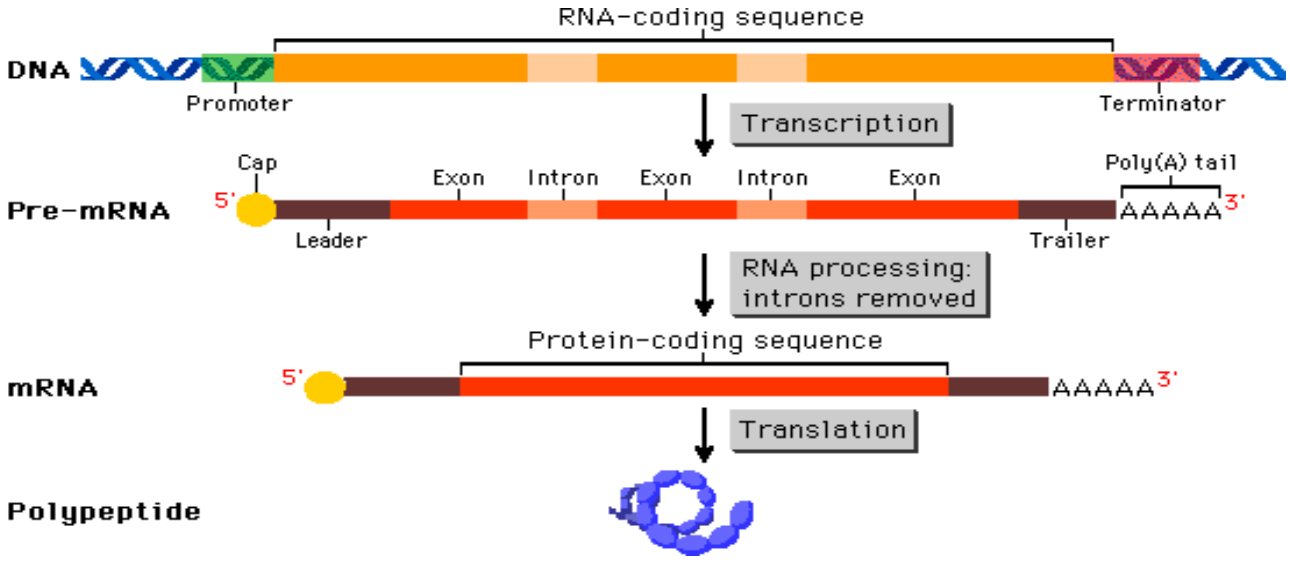
Concept 8: mRNA in Prokaryotes

The sequence of a prokaryotic protein-coding gene is colinear with the translated mRNA; that is, the transcript of the gene is the molecule that is translated into the polypeptide.



Concept 9: mRNA in Eukaryotes

The sequence of a eukaryotic protein-coding gene is typically not colinear with the translated mRNA; that is, the transcript of the gene is a molecule that must be processed to remove extra sequences (introns) before it is translated into the polypeptide.



Most eukaryotic protein-coding genes contain segments called introns, which break up the amino acid coding sequence into segments called exons.

The transcript of these genes is the pre-mRNA (precursor-mRNA).

The pre-mRNA is processed in the nucleus to remove the introns and splice the exons together into a translatable mRNA. That mRNA exits the nucleus and is translated in the cytoplasm.

Concept 10: Pre-mRNA Processing (Splicing)

Eukaryotic pre-mRNAs typically include introns. Introns are removed by RNA processing in which the intron is looped out and cut away from the exons by snRNPs, and the exons are spliced together to produce the translatable mRNA.

The steps of pre-mRNA splicing (intron removal) are as follows:

- The intron loops out as snRNPs (small nuclear ribonucleoprotein particles, complexes of snRNAs and proteins) bind to form the spliceosome.
- The intron is excised, and the exons are then spliced together.
- The resulting mature mRNA may then exit the nucleus and be translated in the cytoplasm.

(2): Types of DNA damage and type of photoreactivation repair and Mismatch repair.

Types of DNA Damage :

1. 1. Deamination: (C → U and A→ hypoxanthine)
2. Depurination: purine base (A or G) lost
3. T-T and T-C dimers: bases become cross- linked, T-T more prominent, caused by UV light (UV-C (<280 nm) and UV-B (280-320 nm)
4. Alkylation: an alkyl group (e.g., CH3) gets added to bases; chemical induced; some harmless, some cause mutations by mispairing during replication or stop polymerase altogether

5. Oxidative damage: guanine oxidizes to 8-oxo-guanine, also cause SS and DS breaks, very important for organelles

6. Replication errors: wrong nucleotide (or modified nt) inserted

7. Double-strand breaks (DSB): induced by ionizing radiation, transposons, topoisomerases, homing endonucleases, and mechanical stress on chromosomes

type of photoreactivation repair and Mismatch repair.

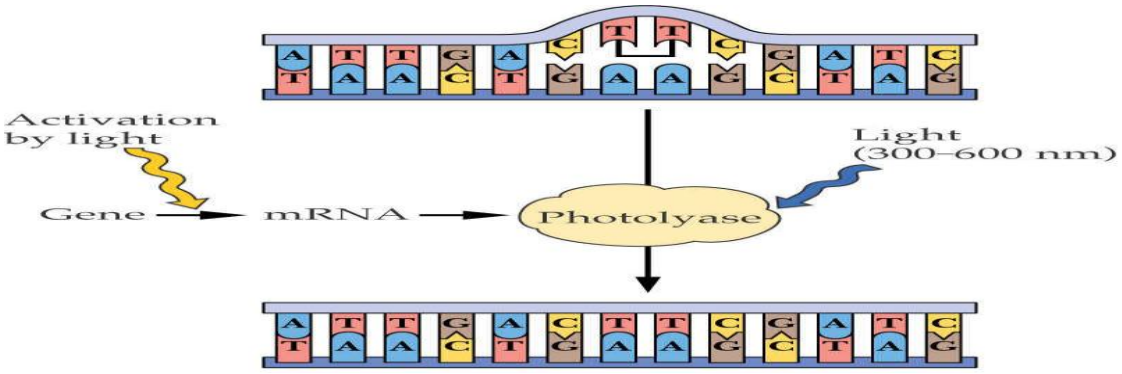
- **Photoreactivation**

1. Light-dependent, UV-A → blue light (360-420 nm)
2. Catalyzed by Photolyases:
    - Enzymes that convert the dimers to monomers

- Use FAD as chromophore and electron donor
- also have another chromophore that acts as antenna
- 3 classes: CPD I and II for T-T dimers, and a 6-4 photolyase for T-C dimers

3. Arabidopsis has CPD II and 6-4 photolyases
4. Arabidopsis also has a photolyase in the chloroplast and possibly one in the mitochondria.

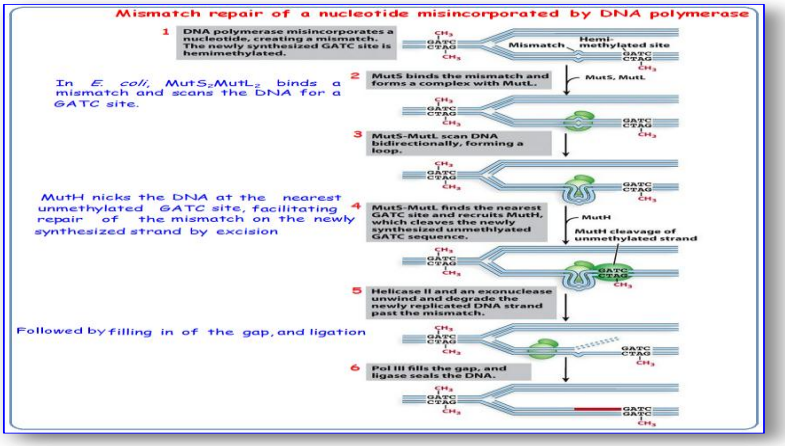
Photolyase gene expression also induced or increased by light.



Mismatch Repair

- Problem: how do cells know which is the right template strand?
- In E. coli, new DNA not methylated right away
  - Mismatch recognized by mutS, then mutL binds and attracts mutH (endonuclease that cleaves mismatch and nearest CTAG that is not methylated)
- Eucaryotes (including Arabidopsis) have mutS and mutL homologues, but no mutH
  - Also have the requisite exonucleases, but not clear how the strand specificity is determined

Mismatch Repair In *E.coli*, A of each GATC is methylated



(3) Difference between Structural and Regulatory Genes.

What are the Similarities Between Structural and Regulatory Genes ?

.. Both Structural and Regulate Genes are code for protein or RNA

- Both Structural and Regulate Genes are made up of nucleus.
- Both Structural and Regulate Genes are important in living organisms.

What is the Different Between Structural and Regulatory Genes

Structural vs Regulatory Genes	
Structural gene is a type of gene that encodes for any type of RNA (except siRNA and miRNA) and protein that are not regulatory proteins.	Regulatory genes are a set of genes that involve the controlling of expression of the structural genes.
Structure	
Structural genes are complex structures.	Regulatory genes are simpler structures.
Function	

Structural genes are encoded for structural proteins and enzymes.	Regulatory genes regulate the transcription of structural genes.

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Third Question : (20 Marks):

(1): Explain: The basic procedures of recombinant DNA technology and its applications.

Recombinant DNA technology is a technology which allows DNA to be produced via artificial means. The procedure has been used to change DNA in living organisms and may have even more practical.

How recombinant technology works ?

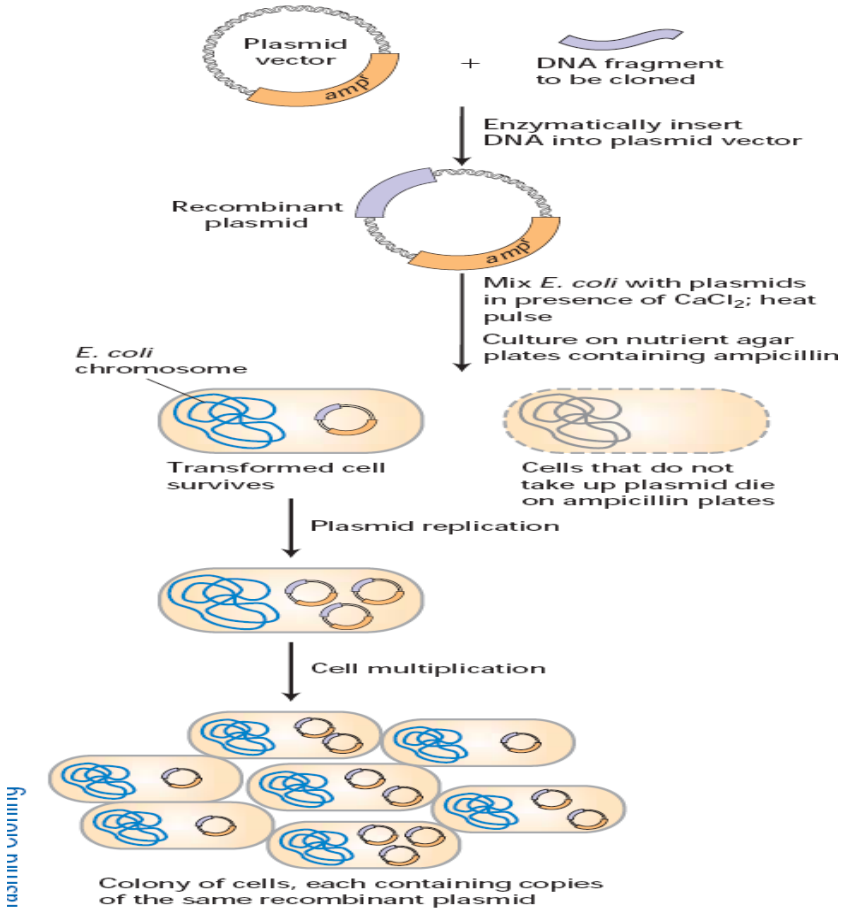
- These steps include isolating of the target gene and the vector, specific cutting of DNA at defined sites, joining or splicing of DNA fragments, transforming of replicon to host cell, cloning, selecting of the positive cells containing recombinant DNA, and either express or not in the end.

Six steps of Recombinant DNA

1. Isolating (vector and target gene)
2. Cutting (Cleavage)
3. Joining (Ligation)
4. Transforming
5. Cloning
6. Selecting (Screening)

The basic procedures of recombinant DNA technology

DNA cloning in a plasmid vector permits amplification of a DNA fragment.



Applications of Recombinant DNA Technology

Analysis of Gene Structure and Expression
Pharmaceutical Products

- Drugs, Vaccines

.Genetically modified organisms (GMO)

- Transgenic plants .Transgenic animal

Application in medicine

(2): Complete The following (Only five Points)

(1) (Decode the mRNA -----Form peptide bonds for protein synthesis.

(2): bind RNA polymerase to the promoter and initiate transcription.

(3) : DNA glycosylase , AP endonuclease , Deoxyribose phosphodiesterase , DNA Polymerase and Ligase.

(4) : Operator Gene

(5) : Operon

(6) : gene only transcribed when need as opposed to a constitutive gene.

(7) : Inducible operon –and Repressible operon.

With my best wishes

Prof .Dr / Mohamed Serag Eldin