

LEARNING OBJECTIVES



By the end of this presentation, you will be able to:

- 1. Define cloning vectors, and identify key examples (e.G., Plasmids, bacteriophages, and viruses).
- 2. Compare the features of different types of vectors (e.G., Plasmids vs. Phages) and evaluate their suitability for pharmaceutical applications.
- 3. Assess the importance of specific vector characteristics (e.G., Replication origin, selectable markers, multiple cloning sites) for successful gene cloning and protein expression.
- 4. Design a practical approach to selecting and using a vector for a pharmaceutical biotechnology application, such as therapeutic protein production.

DR ALA ABUHAMMAD, PHD

Nov-24

GENE CLONING

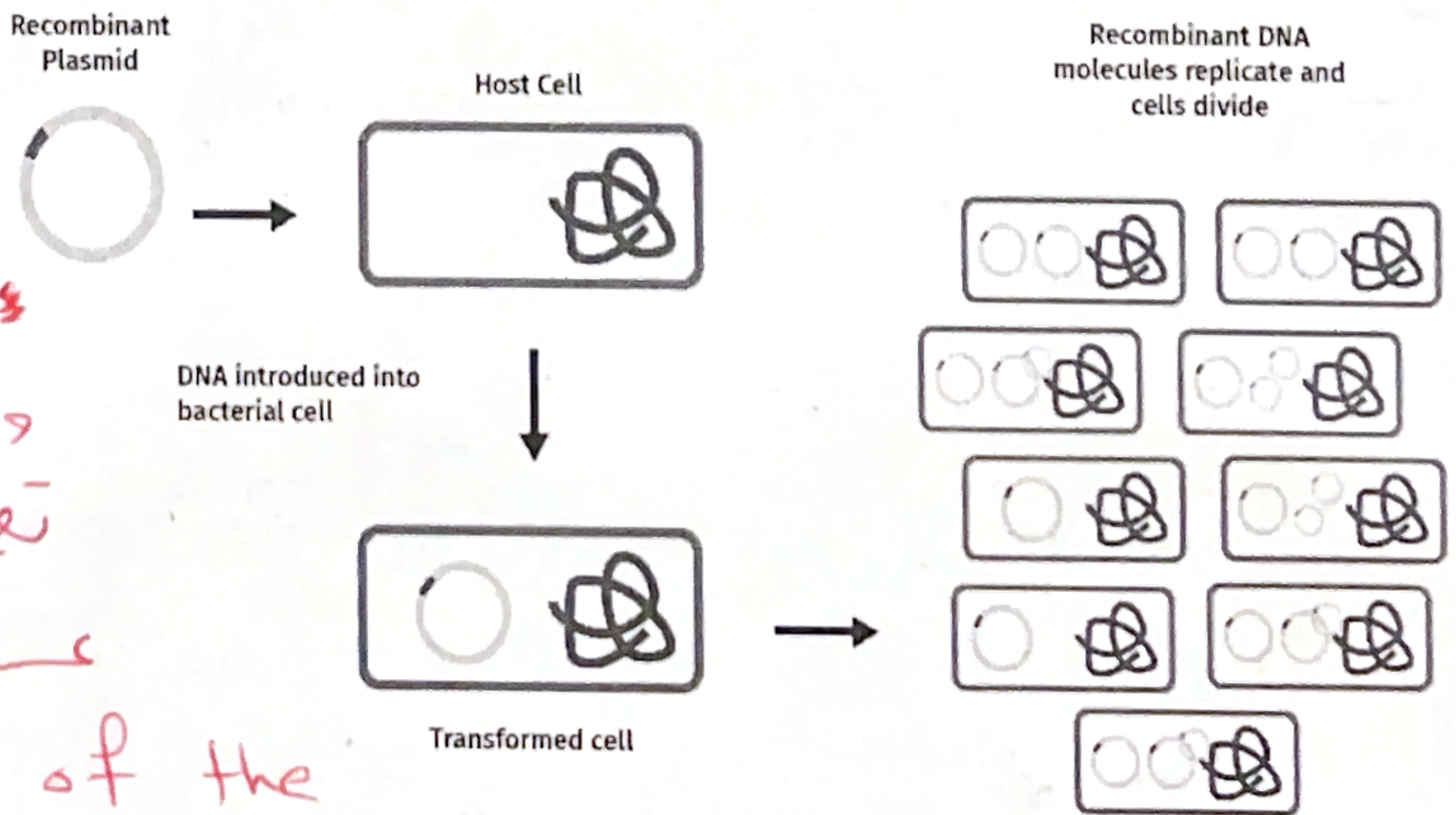


الجامعة الهاشمية
The Hashemite University



Gene cloning in recombinant DNA (rDNA) technology is the process of creating multiple identical copies of a specific gene of interest by inserting it into a suitable vector and propagating it within a host organism (e.g., bacteria, yeast).

الفكرة: إدخال تفاعل
recombinant DNA
to the host cells
host cell replication
عشان يتنقل على
clones or colonies of the
transformed or recombinant cells



Nov-24

تذكير (أخذنا هجلا)

Steps in Gene Cloning in rDNA Technology:

1. Isolation of Gene of Interest:

The gene to be cloned is extracted from the source DNA (e.g., human, plant, or microbial DNA) using restriction enzymes or PCR.

2. Insertion into a Vector:

The isolated gene is inserted into a vector (e.g., plasmid or phage DNA) that can carry the gene into the host cell.

Ligation is performed using DNA ligase to attach the gene into the vector at a cloning site.

3. Introduction/insertion into Host Cell (Transformation):

The recombinant vector is introduced into a host cell (usually *E. coli* for its fast replication and ease of use).

4. Selection of Clones:

Only the host cells that successfully incorporated the recombinant DNA are selected, typically using selectable markers like antibiotic resistance genes.

5. Replication and Expression:

Inside the host, the recombinant DNA replicates, producing many copies of the gene. Under specific conditions, the cloned gene can also be expressed to produce the corresponding protein.

Nov-24

5

Dr Ala Abuhammad, PhD

(اختياره بكل صحيح في ترعاى نجاح العملية كاملة)

اختيار
vector ←

optimization
through process of cloning
VECTOR TYPES FOR CLONING

Vector -it carries recombinant DNA into cells.

Small DNA molecule capable of self replication that are used as cloning vehicle or carrier of DNA fragment.

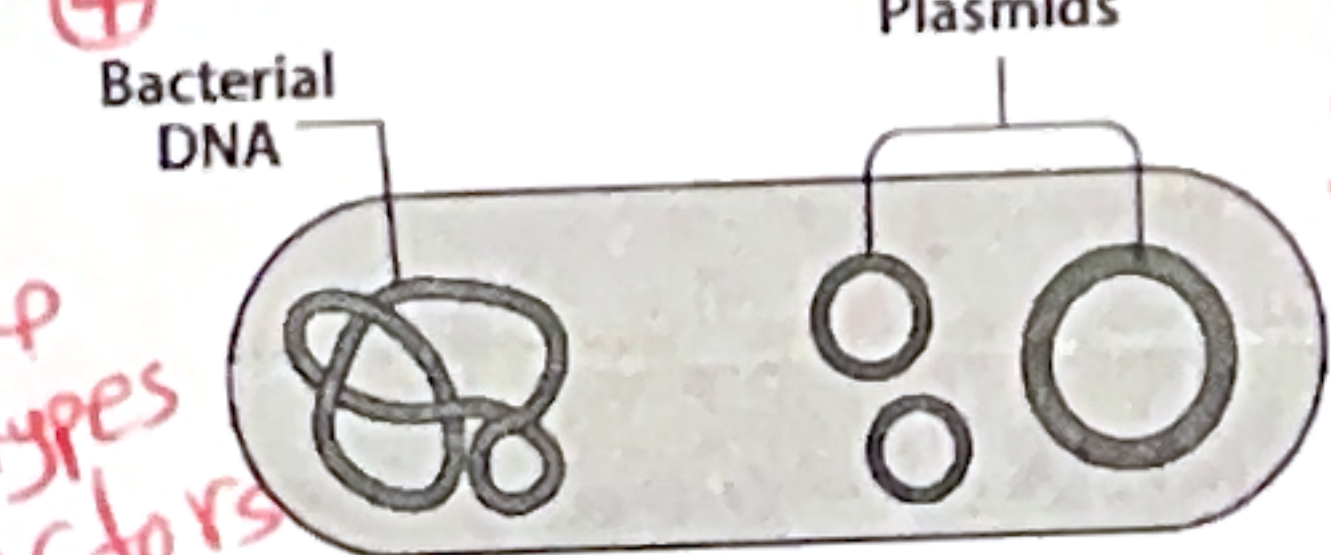
Selection of the vector type depends on host used in the cloning and size of insert

① Plasmids

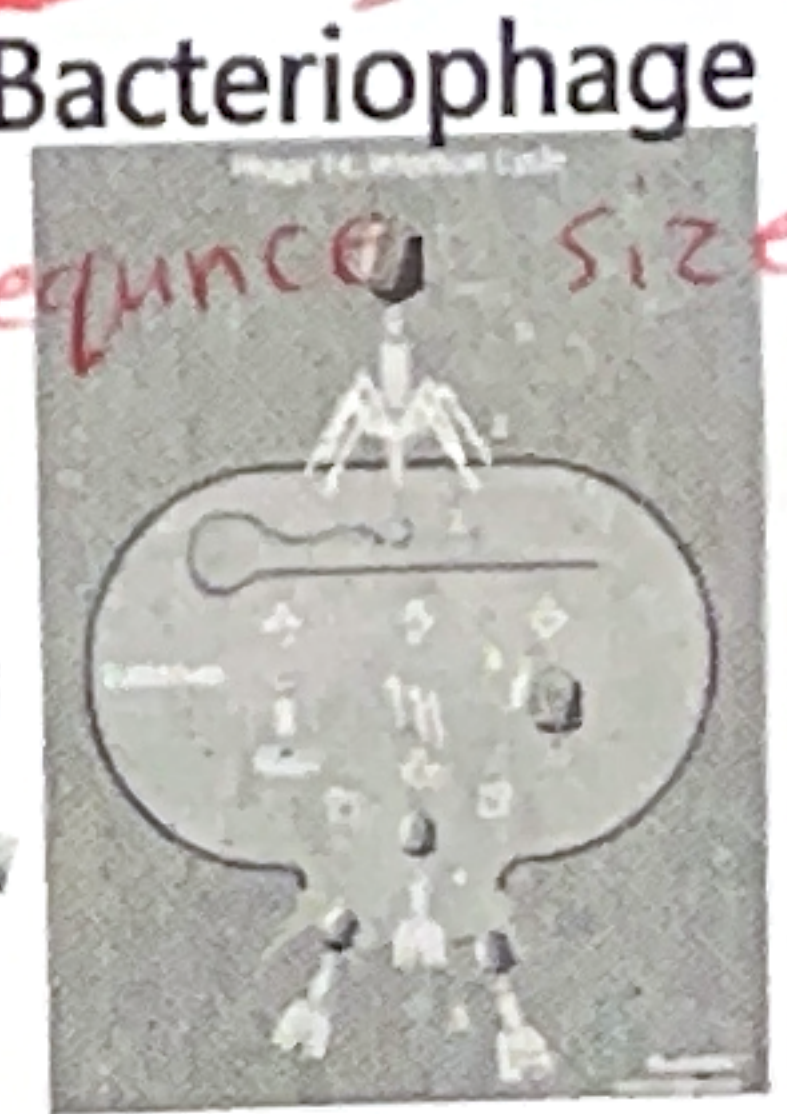
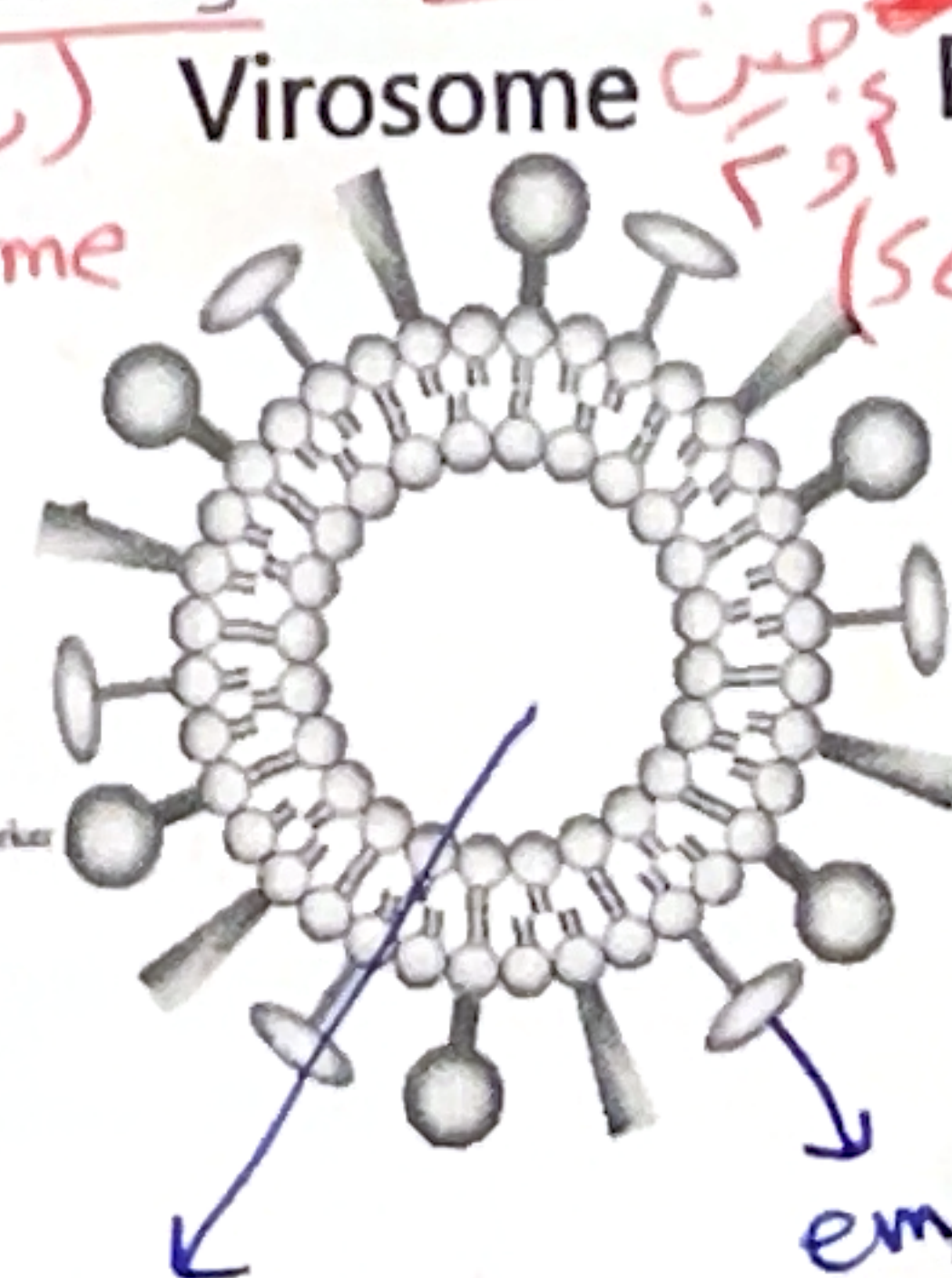
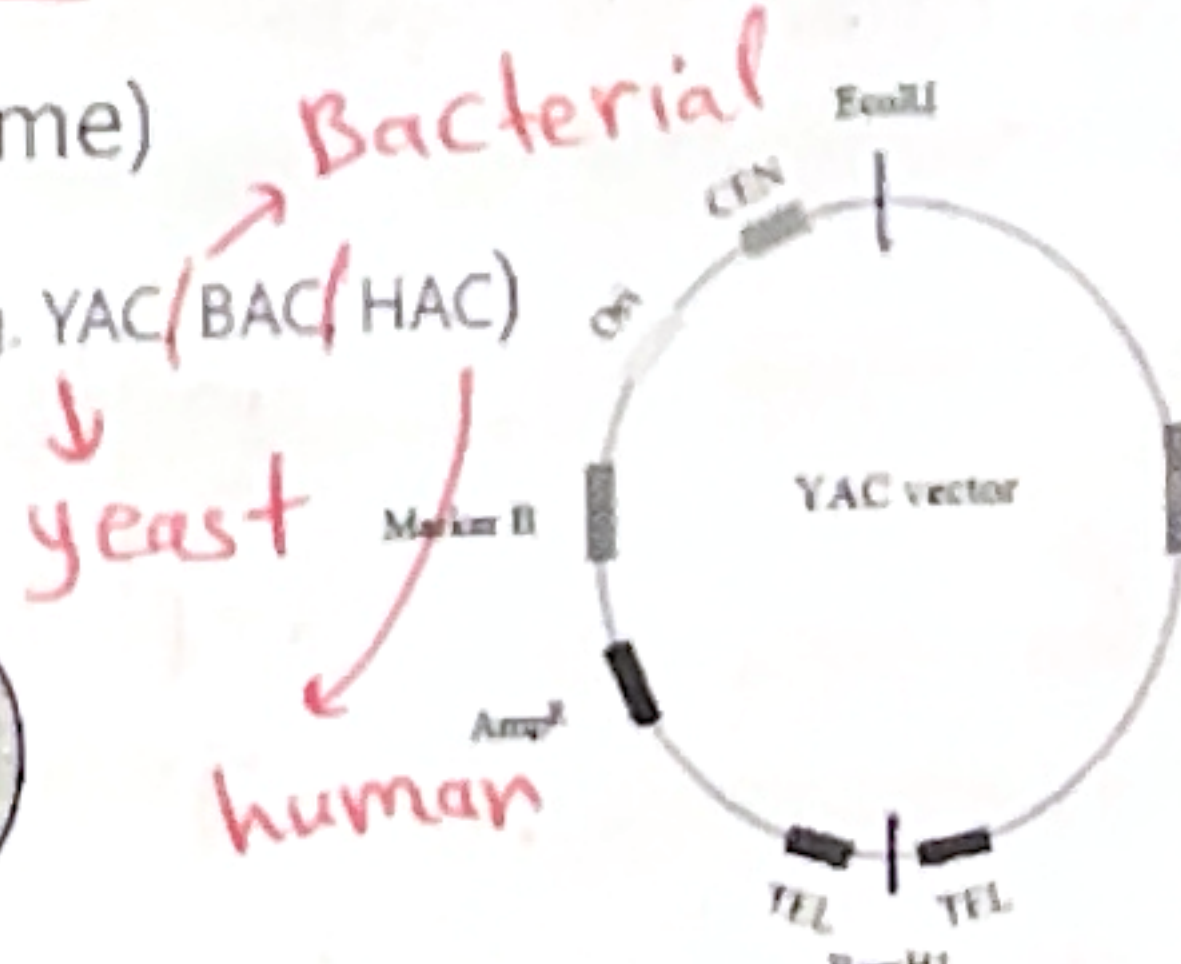
② Phages (e.g. Bacteriophages)

③ Hybrid vectors (e.g. virosome)

④ Artificial chromosomes (e.g. YAC/BAC/HAC)



Dr Ala Abuhammad, PhD



loaded with plasmid

embedded with spikes or proteins
يكون موجودين على سطح الفيروس

عشان هيل لازم نشأه ان ال vector
well not induce an immune reaction

VECTOR TYPES FOR CLONING

Vector Type	Description	Insert Size Capacity	Example/ Application
Plasmids	Circular, double-stranded DNA found in bacteria; used for small insert cloning.	~1-10 kb	Production of insulin or growth hormones in recombinant <u>E. coli</u> .
Phages	Bacteriophages (viruses infecting bacteria) used as vectors for cloning.	~10-20 kb	Generation of DNA libraries for identifying therapeutic targets.
Hybrid Vectors	Combine features of plasmids and phages (e.g., cosmid, virosome), to enhance their functionality.	~35-45 kb	Gene therapy: delivering genes to correct genetic disorders.
Artificial Chromosomes	Synthetic chromosomes (e.g., BACs, YACs) used for large-scale cloning.	Up to 300 kb (BAC), 1 Mb (YAC)	Humanized monoclonal antibodies using transgenic animals.

Dr Ala Abuhammad, PhD

Nov-24

7

ازا يدهم يعملو أبحاث بعلو libraries
و شوفو سؤال phenotype اي بنتج عن هاي ال
DNA sequence vector لهاي العملية
phages

VECTOR CHARACTERISTICS

1 Self-Replication

- Vectors must have an origin of replication (Ori), enabling them to replicate independently within the host cell.

Allows production of multiple copies of the gene of interest.

2 Cloning Site

- Vectors contain multiple cloning sites (MCS) or restriction sites, which are unique sequences recognized by restriction enzymes.

- These sites allow precise insertion of the gene of interest without disrupting essential vector functions.

3 Selectable Marker Gene

- Vectors carry genes like antibiotic resistance (e.g., ampicillin or kanamycin resistance) to help identify and select host cells that successfully took up the vector.
- Selectable markers are critical for screening recombinant cells.

4 Small Size

- Smaller vectors are easier to manipulate and transfer into host cells.
- Reduces metabolic burden on the host, enhancing replication efficiency.

Dr Ala Abuhammad, PhD

Nov-24

8

بعض النظر عن نوع
كثير لازم يكون عندهم هاي الخواص
بعض النظر عن نوع
كثير لازم يكون عندهم هاي الخواص
independent resources
independence from the genetic

فازا كنت عارف انه عندي promoter او operator بهادار vector يساعدوني اتحكم بال expression لل gene of interest ، لكن خصل على yeild معين بيا اياه من ال protein production (ما بيا يكون قليل و لا كثير عالي) + عنسب toxicity لاخلق و تقوت

VECTOR CHARACTERISTICS



الجامعة الهاشمية
The Hashemite University



5 Stability

- The vector should be stable within the host and not undergo structural alterations or degradation.
- Prevents loss of the gene of interest during replication.

6 Replication in Host Cell

- The vector's origin of replication determines compatibility with specific hosts (e.g., plasmids for bacteria, yeast artificial chromosomes for yeast).
- Ensures high copy numbers or controlled replication as needed.

7 Control Elements for Gene Expression

- Includes promoters, operators, ribosome binding sites, and terminators for regulated expression of the gene of interest.
- Allows flexibility for induced or constitutive gene expression.

8 Easily Isolated and Purified

- Vectors should be simple to extract and purify from host cells using standard molecular biology techniques.

Dr Ala Abuhammad, PhD

Nov-24

9

مثلا (artificial chromosomes) ما بنفع استخدمهم في حالة البكتيريا (ما راع يصير الكروموسوم expression من البكتيريا) ، لانه يكون كثير developed اكثر من البكتيريا (ال functions داخل البكتيريا ما راع تقدر تدعى expression لهدول الكروموسوم)

PROMOTERS AND CONTROL ELEMENTS



الجامعة الهاشمية
The Hashemite University



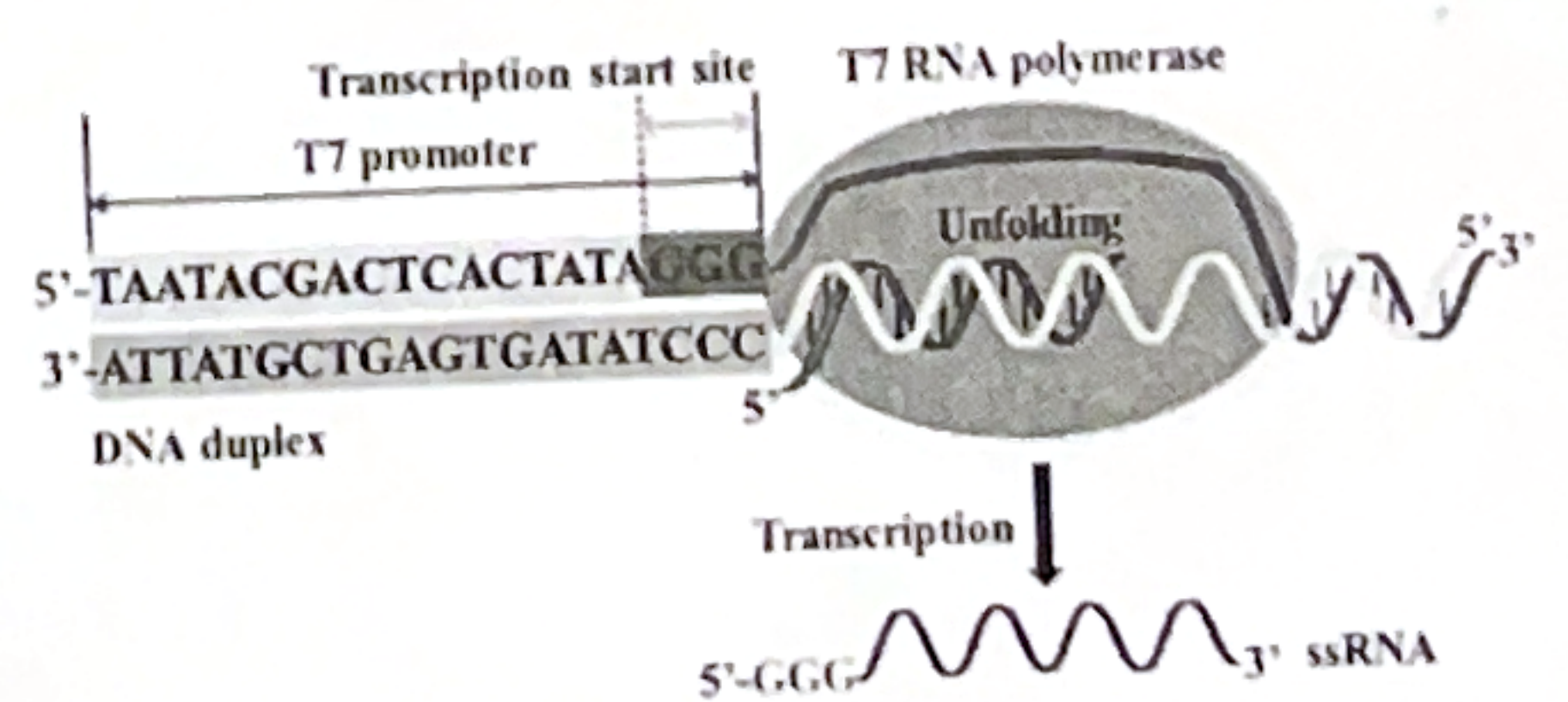
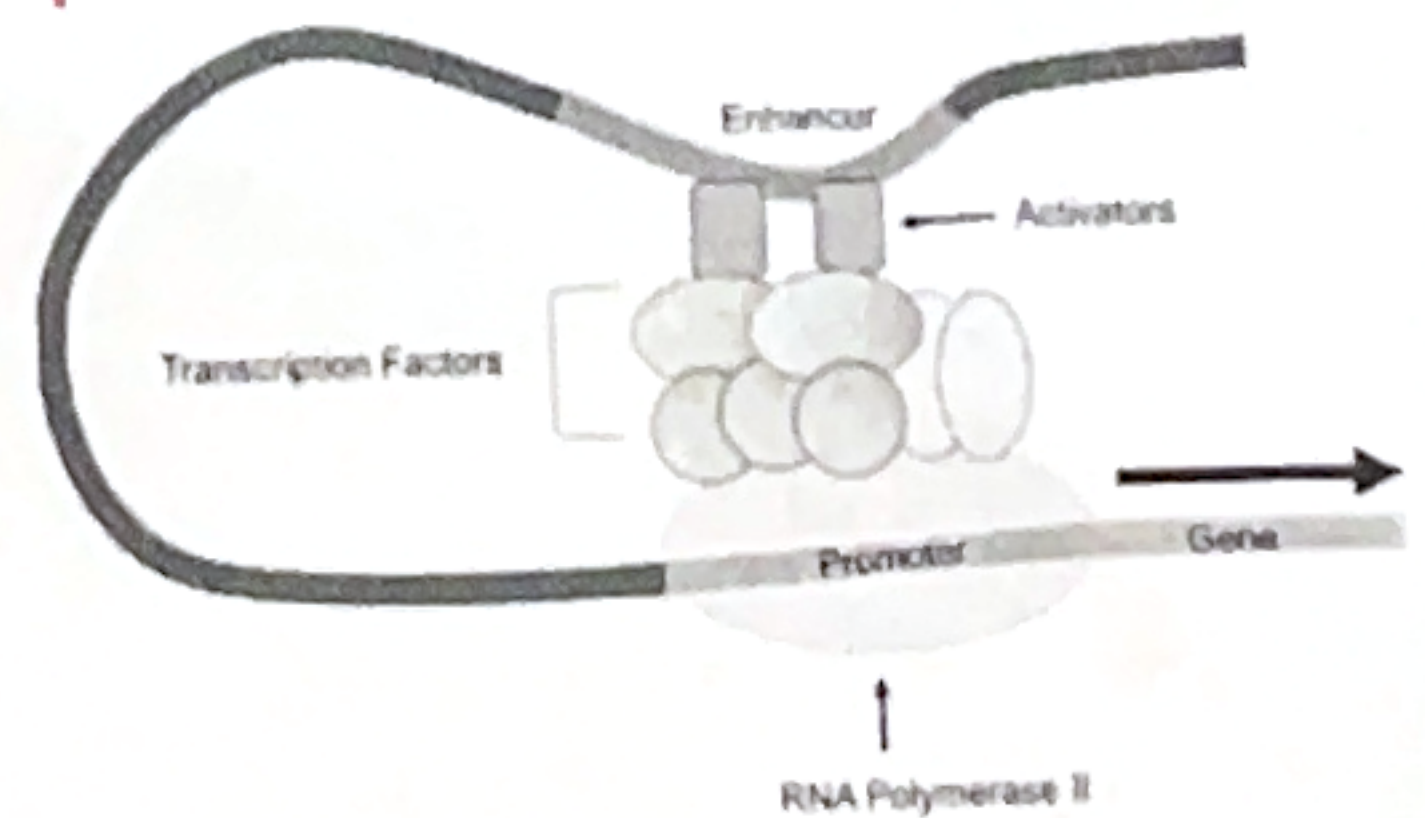
expression rate of the gene regions بتعلقهم trans factor ، بزياد من

- Promoters regulate gene transcription in host cells.
- Types of Promoters:**
 - Strong bacterial promoters (e.g., T7) for recombinant protein overexpression.
 - Recognized by the T7 RNA polymerase (not the host's RNA polymerase).
 - Extremely strong, leading to high levels of transcription.
 - Typically used for overexpression of recombinant proteins in bacterial systems (e.g., *E. coli*).
 - Produces large amounts of therapeutic proteins, such as insulin or monoclonal antibodies.
 - Used for industrial production of enzymes, vaccines, and research proteins.

Control on expression rate

- Mammalian promoters (e.g., CMV) for eukaryotic applications.

Dr Ala Abuhammad, PhD



Nov-24

10

- Other Control Elements:
- Operators are incorporated into expression vectors to regulate the transcription of the gene of interest.
 - They allow conditional expression, meaning the gene is expressed only under specific conditions (e.g., presence or absence of an inducer or repressor).
 - Repressors: Proteins that bind to the operator and prevent transcription.
 - Activators: Proteins that enhance transcription by binding to the operator or adjacent sequences, increasing RNA polymerase activity.
 - By using a controlled operator system, therapeutic proteins can be produced in bacteria or mammalian cells only when needed, preventing wastage of cellular resources or toxic protein accumulation.
- Ribosome binding sites (RBS): Ensure efficient translation.
 - Adjusting Sequence: Altering the RBS sequence can fine-tune translation efficiency, controlling the rate of protein synthesis.
 - An optimized RBS can increase protein production by improving ribosome recruitment and alignment with the start codon.

Dr Ala Abuhammad, PhD

Nov-24

11

برقوبة، راتكم فيهم

using certain agents

بصحت أنكم أو أصب كمة البروتين

translation

HOST SPECIFICITY AND COMPATIBILITY



- Vectors must be compatible with the host for successful gene expression.
- Common hosts and suitable vectors:
 - Bacteria: Plasmids, phages (e.g., E. coli).
 - Yeast: YACs for larger inserts.
 - Mammalian Cells: Viral vectors for therapeutic applications.
- Transformation Methods:
 - Heat shock and electroporation for plasmids.
 - Viral delivery for mammalian cells.

host cells

كيف يتم كمة
transformation of the vector
into the host

Dr Ala Abuhammad, PhD

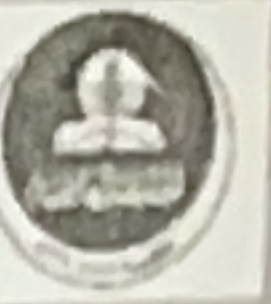
Nov-24

12

SELECTABLE MARKER GENES



الجامعة الهاشمية
The Hashemite University



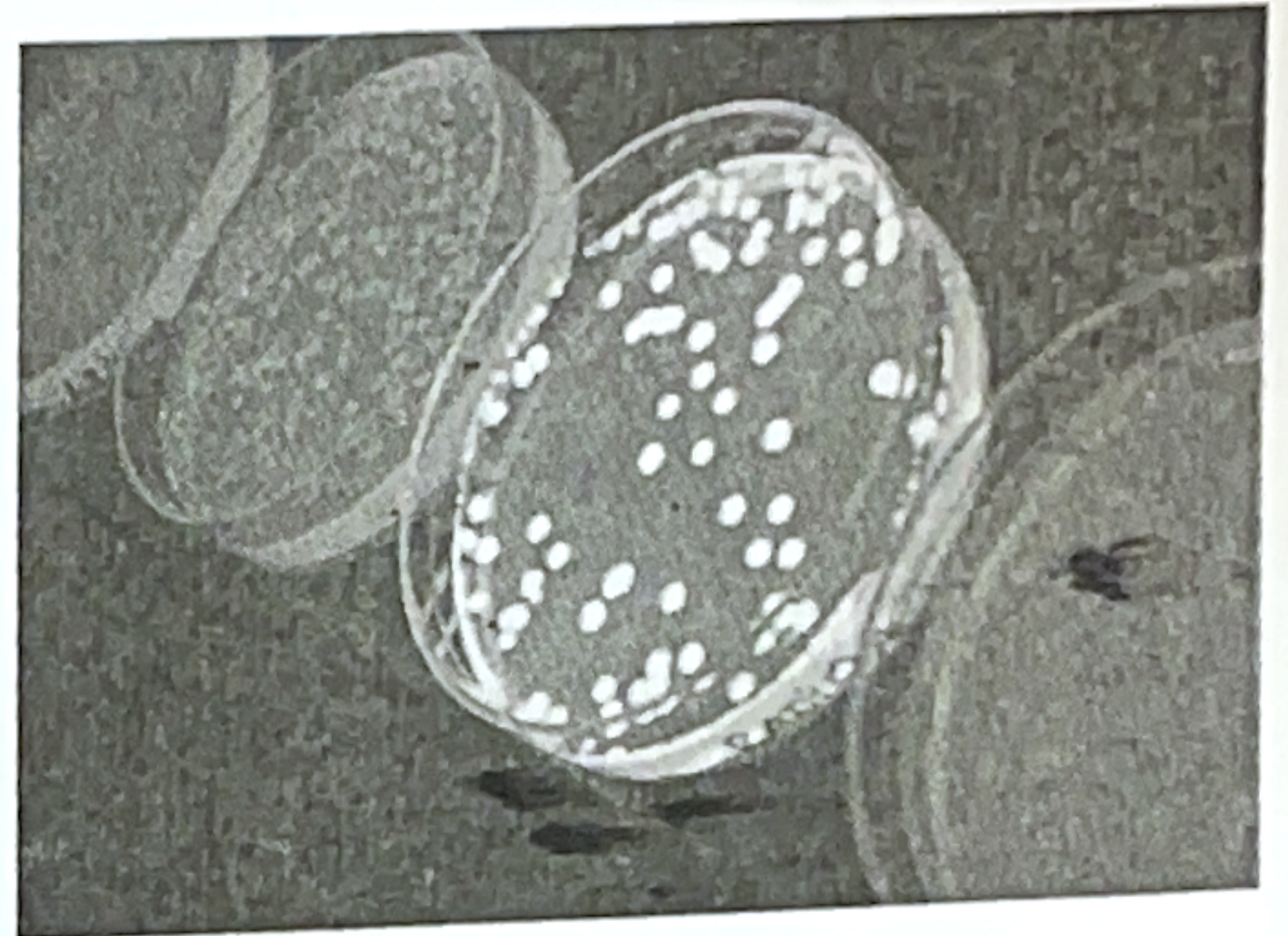
- Selectable markers help differentiate transformed from non-transformed cells.

Common Types:

- Antibiotic Resistance: E.g., ampicillin resistance gene.
- Reporter Genes: E.g., GFP (green fluorescent protein) for visual confirmation.

Selection Methods:

- Antibiotic plates for bacterial colonies.
- Fluorescence or colorimetric assays.



Nov-24

13

Dr Ala Abuhammad, PhD

← عشان هيلك vector لازم يكون عندهم خاصية او قابلية انه انت تضيف لهم جينات معينة تساعد في عملية selection من antibiotic resistance gene, ازا كان ال vector فيه

antibiotic resistance gene

antibiotic resistance gene

COLONY SCREENING

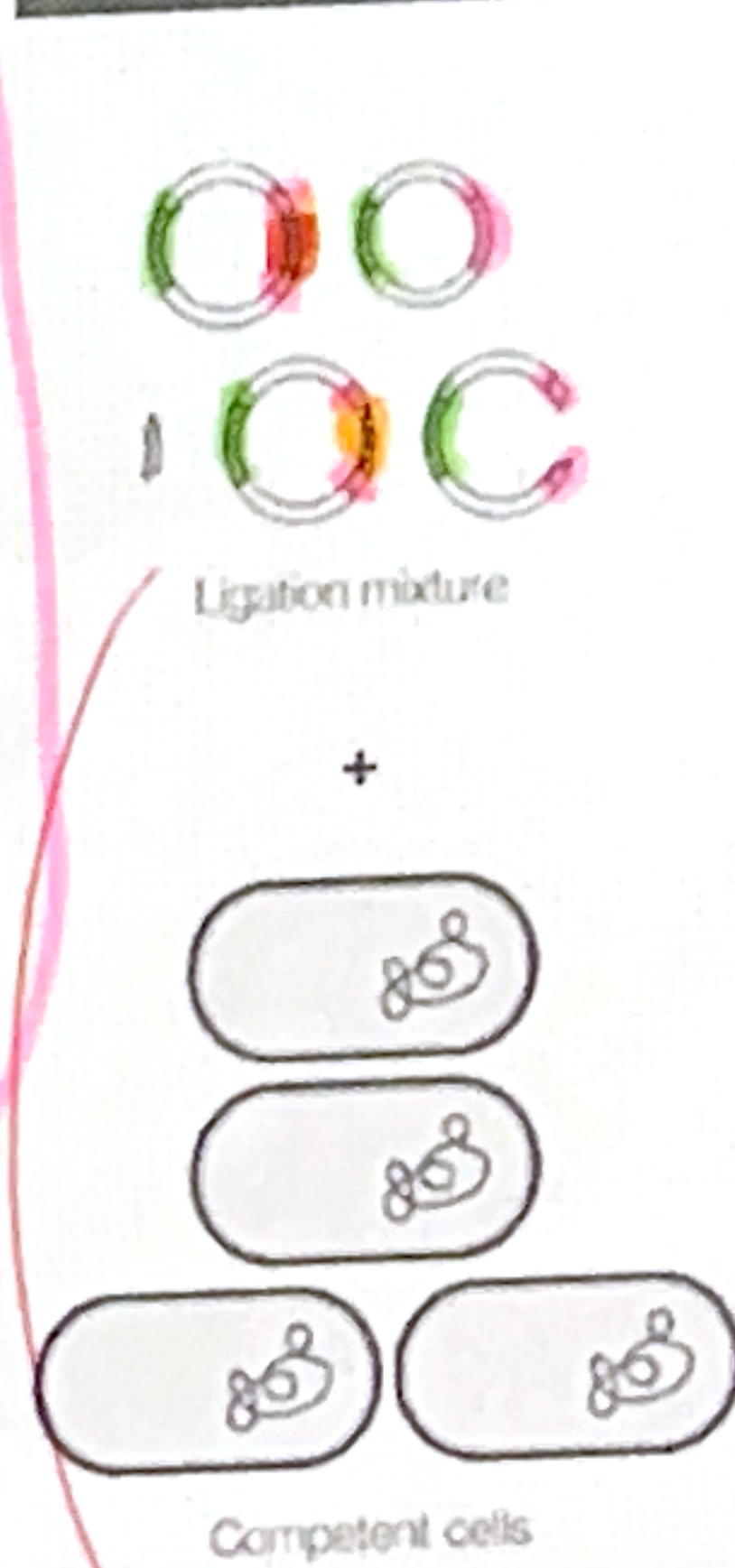
الخلايا اللي حار بهم successful transform resistance, ازا حطيت عليهم ما يكونو resistance, هاد الجين المسؤول عن antibiotic resistance successful transformation ligation and transfection of the vector into the cell



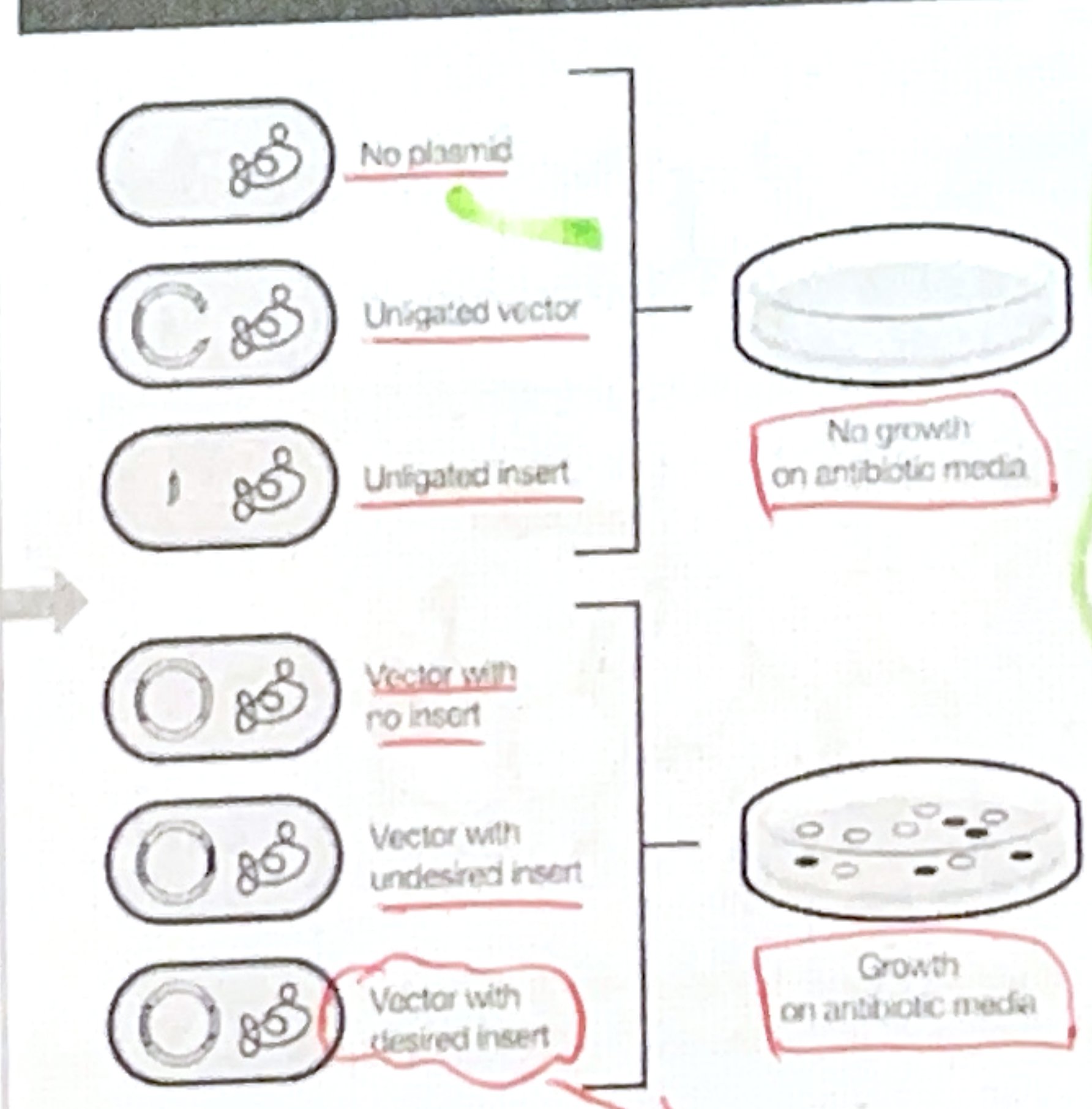
الجامعة الهاشمية
The Hashemite University



Transformation



Cell plating



Further analyses

- Blue/white screening
- Positive selection
- Restriction digestion
- Colony PCR
- Sanger sequencing

بالتالي كالتحليل هاي الخلايا antibiotic, unsuccessful transformed cells وراح يضل عاين الخلايا اللي حار بهم successful transformation

Nov-24

15

Dr Ala Abuhammad, PhD

هون عنا اختيارات يدي ممكن بغير مثلك ارتباط حيث فاطمة ما حار ligation (للجين مع البلازميد)

بس هاد الناتج

sequence