Biotechnological Applications of Recombinant DNA

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I Chapter I: Expression vectors

1. Introduction

What is a vector?

A vector is a small piece of DNA molecule, which is used as a vehicle to artificially carry foreign genetic material into host cell, where it can be replicated and/or expressed. A vector containing foreign DNA is termed as recombinant DNA. The purpose of a vector is typically to isolate, multiply, or express the insert in the target cell. Useful vectors have three main features: (i) An origin of replication (Ori), which is a specific DNA sequence at which DNA replication is initiated. The essential feature of a vector is that it can replicate autonomously in a host species, usually bacteria. The origin is therefore absolutely essential for the amplification of the vector inside a bacterial host. (ii) Presence of a multiple cloning site (MCS), a region with multiple useful restriction enzyme sites to make a compatible digest of the vector and DNA fragment. (iii) A selectable marker, which is a method of allowing hosts (usually bacteria) containing the vector to be readily identified and purified. The insert contains a selectable marker which allows for identification of recombinant molecules. An antibiotic marker is often used so that a host cell without a vector dies when exposed to a certain antibiotic, and the host with the vector will live because it is resistant. Cloning vector and expression vector are two types of vectors, used in recombinant DNA technology. A cloning vector is a small piece of DNA which can be stably maintained within a host cell, and used to introduce genes into cells while obtaining numerous copies of the insert. A vector designed specifically for the transcription and protein expression of the transgene in the target cell is called expression vector, and generally have a promoter sequence that drives expression of the transgene *[4]*[4]*.

2. Objectives

In this chapter, the student will be able to :

- Understand the concept of vectors and their role in genetic engineering, particularly in the context of recombinant DNA technology.
- Differentiate between cloning vectors and expression vectors, and comprehend the specific features and applications of each type.
- Analyze the significance of CRISPR-Cas9 technology in biotechnology and its potential applications in genetic engineering and gene editing.
- Understand the concept of transformation and its significance in genetic engineering processes.

3. Definition of expression vectors

Cloning vectors provide a backbone for the DNA insert to be reproduced and propagated in bacteria; however, these vectors are only useful for storing a genetic sequence. By themselves, they are incapable of allowing for transcription and translation of the gene into a functional protein product. The expression vectors are vectors, which act as vehicles for DNA insert and also allow the DNA insert to be expressed efficiently. They are special

types of cloning vectors containing the regulatory sequences necessary to allow the transcription and translation of a cloned gene or genes. These vectors contains several key elements *[5]*[5]*, including:

- **Promoter:** This is a DNA sequence that initiates the transcription of the inserted gene into messenger RNA (mRNA). Different promoters can be used to control the level and timing of gene expression.
- **Transcription Stop Site:** This is where transcription of the GOI ends. It is signaled by the polyadenylation signal (PolyA), which ensures proper mRNA termination.
- **Tags :** Help in identifying and purifying the protein of interest once it has been expressed and isolated. Common tags include His-tags (polyhistidine tags), FLAG tags, Myc tags, and GFP (Green Fluorescent Protein) tags.
- **Polyadenylation Signal (PolyA):** This sequence signals the termination of mRNA transcription. It ensures that the mRNA is properly processed and stable.

Expression vector (cf. 119_Cloning%20vectors.pdf)

4. Types of expression vector

The choice of classic cloning vectors depends on size of the insert and application *[5]*[5]*.

4.1. Plasmid

Plasmids are extra chromosomal double-stranded DNA sequences found in the cytoplasm of microbes and capable of replication using the host cell's replication machinery. They are generally circular and stable genetic entity that can replicate itself autonomously, independent of the chromosomal DNA of the host organism. They are found widely in many bacteria, for example in E. coli, but may also be found in a few eukaryotes, for example in yeast such as Saccharomyces cerevisiae. Plasmids usually carry at least one gene, and many of the genes that plasmids carry are beneficial to their host organisms. Although they have separate genes from their hosts, they are not considered to be independent life. Plasmids contain genes that enhance the survival of an organism, either by killing other organisms or by defending the host cell by producing toxins. Multiple plasmids can coexist in the same cell, each with different functions. In other words, plasmids can only co-occur in a bacterium if they are compatible with each other. They are incompatible if they have the same reproduction strategy in the cell. An incompatible plasmid will be expelled from the bacterial cell.

Moreover, plasmids can be eliminated from bacterial cells by a process called curing, which may take place spontaneously or by various curing treatments, such as acridine dyes, ultraviolet (UV) and ionizing radiation, thymine starvation and growth above optimal temperatures that inhibit plasmid replication but do not affect bacterial chromosome replication and cell reproduction. They have three key points: The origin of replication, which is used to indicate where DNA replication is to begin; The selection marker gene, which is used to distinguish cells containing the plasmid from cells that do not contain it; and the cloning site, a site in the plasmid where the DNA is inserted.

The use of plasmids as a expression vector is ones most commonly used. Most general plasmids may be used to clone DNA insert from 5 to 15 kb in size. One of the earliest commonly used expression vectors is the pBR322 plasmid. Many plasmids have high copy number, for example pUC19 which has a copy number of 500-700 copies per cell and high copy number is useful as it produces greater yield of recombinant plasmid for subsequent manipulation. However low-copy number plasmids may be preferably used in certain circumstances, for example, when the protein from the cloned gene is toxic to the cells. A plasmid is isolated from the bacterial cell at one site by restriction enzyme. The cleavage converts the circular plasmid DNA into a linear DNA molecule. Then the two open ends of

linear plasmid are joined to the ends of the foreign DNA to be inserted with the help of enzyme DNA ligase. This regenerates a circular hybrid or chimeric plasmid, which is transferred to a bacterium wherein it replicates and perpetuates indefinitely. Plasmids often confer antibiotic resistance to the bacteria, so only bacteria containing the vector will survive treatment with antibiotics. Antibiotic resistance is often used as marker, an example is the beta-lactamase gene which confers resistance to the penicillin group of beta-lactam antibiotics like ampicillin. The plasmid vectors also possess promoter sequences near the MCS for efficient expression of gene of interest. The most widely used plasmid in *E. coli* expression systems are presented in the table 1.

Plasmid Name	Promoter	Origin of Replication	Replicon Type	Copy Number
pUC19	Lac promoter (lacUV5)	pMB1 (ColE1-like) origin	Theta replication	High (10-100s)
pBR322	Tet promoter (Tn5)	pMB1 (ColE1-like) origin	Theta replication	Medium (10-20)
pET28a	T7 promoter	p15A origin	Rolling-circle	Low (1-2)
pBAD24	AraC-PBAD promoter	pSC101 origin	Rolling-circle	Low (1-2)
PACYC-Duet1	T5 promoter	p15A origin	Rolling-circle	Low (1-2)
pGEX-6P-1	Tac promoter	pMB1 (ColE1-like) origin	Theta replication	Medium (10-20)
pET21a	T7 promoter	pBR322 origin	Theta replication	High (10-100s)
PRSFDuet-1	T7lac promoter (T7/lacO)	p15A origin	Rolling-circle	Low (1-2)
PMAL-c2X	MalE promoter	pBR322 origin	Theta replication	High (10-100s)

Tableau 1 Table 1. E. coli expression plasmids.

✎***Remarque*** **:**

E. coli is known for its rapid growth rate. Under optimal conditions, it can double in number every 20 to 30 minutes. Its fast growth and well-characterized makes it suitable for large-scale protein production.

4.2. Yeast expression vectors

Yeasts, eukaryotic unicellular fungi, contribute a great deal to the study of molecular genetics. They are popular organisms to clone and express DNA in because they are eukaryotes, and can therefore splice out introns. A species of yeast, *Saccharomyces cerevisiae* has been an important model system for biological research because its entire genome has been base sequenced. It is used as a reference to human and other higher eukaryotic genes. This is because the basic cellular mechanics of replication, recombination, cell division and metabolism are generally conserved between yeast and larger eukaryotes, including mammals. Three expression vectors used for the production of intra-extra proteins : **Yeast episomal plasmids** (YEps), **Yeast integrating plasmids**(YIps), **Yeast artificial chromosomes** (YACs), **Yeast replicative plasmids** (YRps), **Yeast centromere plasmids** (YCps), and **Yeast linear plasmid** (YLps).

Example of *Pichia pastoris* Expression Systems

The expression vectors for *Pichia pastoris* contain the following elements, which is presented in Figure 4:

- Strong promoter: such as methanol inducible AOX1 promoter, or strong constitutive promoters (such as GAP1).
- Signal peptide to direct protein secretion: such as α-factor signal peptide.
- Homologous sequences for chromosomal integration at the AOX1 locus.
- A double recombination happened between the AOX1p and AOX1 regions of the vector where the homologous segments DNA results in the insertion of GOI.

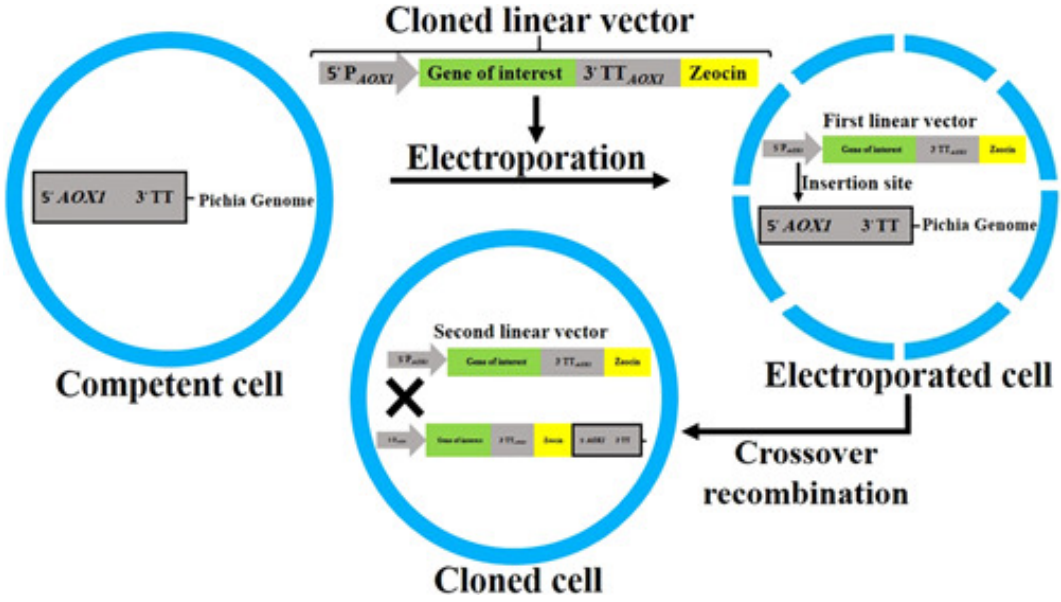

Figure 4. Pichia pastoris expression vector.

Though S. *cerevisae* is successfully used to produce recombinant proteins for human, it has major drawbacks.

- The level of protein production is low.
- There is the tendency for hyper glycosylation resulting in change of protein function.
- Proteins are often retained in periplasm, increasing time and cost for purification.
- It produces ethanol at high cell densities, which is toxic to cells.
- *P. pastoris* is a methylotrophic yeast that is able to utilize methanol as a source of carbon and energy.
- Glycosylation occurs to a lesser extent and the linkages between sugar residues are of the α-1,2type.
- *P. pastoris* strain was extensively engineered with the aim of developing a "humanized" strain that glycosylate proteins in a manner identical to that of human cells.

⊕*Complément* :

- A YAC is designed to clone a large segment of DNA (100kb), which is then maintained as separate chromosome in the host yeast cell.
- Yeps are small (2μm), circular pieces of DNA that exist independently of the yeast cell's chromosomal DNA.
- A Yip cannot replicate as a plasmid as it does not contain the 2 um of plasmid.
- YRps are able to multiply as independent plasmids due to the presence of an origin of replication.

5. Exercice : How are plasmids eliminated from bacterial cells through curing treatments?

[solution n°1 p. 18]

Plasmids elimination?

- O By using antibiotics
- O By exposing them to optimal temperatures
- O By a process involving restriction enzymes and ligase
- O By letting them undergo spontaneous elimination

6. Exercice : Which yeast expression vector type can replicate independently as plasmids?

[solution n°2 p. 18]

Yeast expression vector like plasmids?

- O YEps
- O YIps
- O YACs
- O YRps

7. Transformation methods

How the transformation works?

The process of transferring the recombinant vector into cells usually referred by three different terminologies namely transformation, transfection and transduction. Of these word transformation commonly used.

Transformation: The introduction of any DNA molecule into any living cell.

Transfection: The introduction of purified phage DNA molecules into a bacterial cell. Transduction: The movement of genes from a bacterial donor to a bacterial recipient with the use of a phage as the vector.

Recombinant DNA technology involves the manipulation of DNA molecules to introduce foreign DNA into host organisms, enabling the expression of specific genes or the production of desired proteins.

The specific method used will depend on the nature of the DNA being introduced and the properties of the host cell. Herein, we describe the most used methods as follow:

Transformation methods of recombinant DNA (cf. 211011401.pdf)

7.1. Physical methods

- Electroporation as shown in Figure
- Microinjection
- Liposome mediated gene transfer
- Silicon carbide fibre mediated gene transfer
- Ultrasound mediated gene transfer

7.2. Chemical methods

- PEG mediated gene transfer
- Calcium chloride
- Dextran mediated gene transfer
- Lithium acetate

7.3. Biological methods

- Lentivirus
- Adenovirus
- Liposome

8. Exercice : Which biological method is included in transformation methods of recombinant DNA?

[solution n°3 p. 18]

Transformation

- O Lentivirus
- O Ultrasound mediated gene transfer
- O Lithium acetate
- O Adenovirus

9. Exercice : Transformation method

[solution n°4 p. 18]

The method that involves the introduction of purified phage DNA molecules into a cell is called .

10. Advanced technology in recombinant DNA Crispr (Cas9)

CRISPR, or Clustered Regularly Interspaced Short Palindromic Repeats, is an integral part of a bacterial defense system that was first identified in *E. coli* in the late 1980s. By comparative genomic analysis among different species Jensen et al. in 2002 named the repeat sequences as CRISPR and also defined the common characteristics of CRISPR: (i) its location in intergenic region, (ii) contain multiple short direct repeats (21–37 bp) with little sequence variation, (iii) repeats are interspaced with similar sized non-repetitive nonconserved sequences, (iv) a leader sequences of 300–500 bp were located one side of the repeat clusters (Figure 5). The repeat sequences and leader sequences are conserved among the species. It is also the basis of the CRISPR-Cas9 system. The CRISPR molecule is made up of short palindromic DNA sequences that are repeated along the molecule and are regularly-spaced.

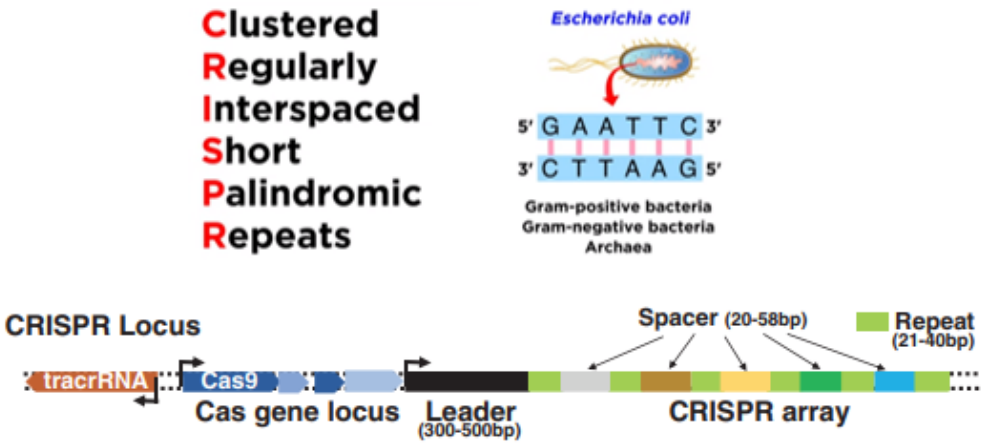

Image 1 Figure 5. Structure of Crispr region in E. coli

Between these sequences are “spacers”, foreign DNA sequences from organisms that have previously attacked the bacteria. The CRISPR molecule also includes CRISPR-associated genes, or Cas genes. These encode proteins that unwind DNA, and cut DNA, called helicases and nucleases, respectively. The CRISPR immune system protects the bacteria from repeated virus attacks through three steps *[6]*[6]*:

10.1. Production of Crispr RNA

When a bacterium survives a viral infection, it captures a small piece of the viral DNA and incorporates it into its own genome, which called adaptation. This process is known as adaptation. The captured viral DNA is stored in the bacterium's genome in the form of short, unique sequences known as CRISPR arrays.

The CRISPR arrays undergoes transcription, including spacers and Cas genes creating precursor CRISPR RNA (pre-crRNA). The resulting single-stranded RNA is called pre CRISPR RNA, which contains copies of the invading viral DNA sequence in its spacers. Then the protein Cas 9 gets involved. Cas refers to Crispr-associated nuclease proteins, and as we know, nucleases are enzymes that are capable of cleaving DNA at specific nucleotide linkages.

In particular, Cas 9 is one of the nucleases found in *Streptococcus pyogenes*, which is one of the most used Crispr-associated nuclease proteins. Along with Cas 9, Tracr RNA, which have a section complementary to the palindromic repeats and can anneal with it. So far, each spacer and palindromic repeats end up with a complex containing a segment of pre-Crispr RNA, tracer RNA, and Cas 9 protein. Then another enzymes called ribonucleases (RNAIII) cleave the strand in between these complexes leaving complexes called effector complexes making the bacteria ready for defending against the invaders. If these complexes encounters with a section of viral DNA that has a sequence, which is complementary to this crRNA, the nuclease enzyme will coordinate, and it recognizes a short sequence unique to the viral genome called a protospacer adjacent motif (PAM), where it will snip a few base pairs upstream from the PAM and cut where it will neutralize the virus and preserve a small part for another invention.

This video is the introductory video of my CRISPR playlist which tells you the story that how CRISPR was discovered.

10.2. Targeting The Crispr RNAs

Scientists have adapted its components into a biotechnology tool for editing DNA. Jennifer Doudna and Emmanuelle Charpentier proposed genome editing using the CRISPR-Cas9 system in 2012 and were awarded the Nobel prize in chemistry in 2020. Single guide RNA (sgRNA) can be synthesized in the lab and used to cleave DNA in humans and other animal species. Cas9 protein along with sgRNA can be used to edit DNA at precise

locations. Synthesize gRNA with complementary sequence and insert into cell along with Cas9 protein. Cas9 will read DNA until it finds appropriate sequence with PAM sequence, bind, and cleave at desired location.

PAM: PAM is short for proto-spacer adjacent motif. It is usually a three-nucleotide sequence consisting of 5'NGG-3' in which the N represents any nucleotide (A, C, G, or T) followed by two guanine (G) nucleotide bases. In humans, PAM motifs occur approximately every 50 bases or less.

When the guide RNA perfectly aligns with the target DNA, the RNA and DNA will form a DNA-RNA helix. This binding event activates Cas9's nuclease, or DNA-cutting, activity. It makes specific cuts in the DNA at a position three nucleotides upstream from the PAM site. Two active sites (regions where molecules bind to undergo chemical reactions) on the nuclease domain of Cas9 generate the cuts and cleave both strands of the DNA double helix, resulting in a double-stranded DNA break.

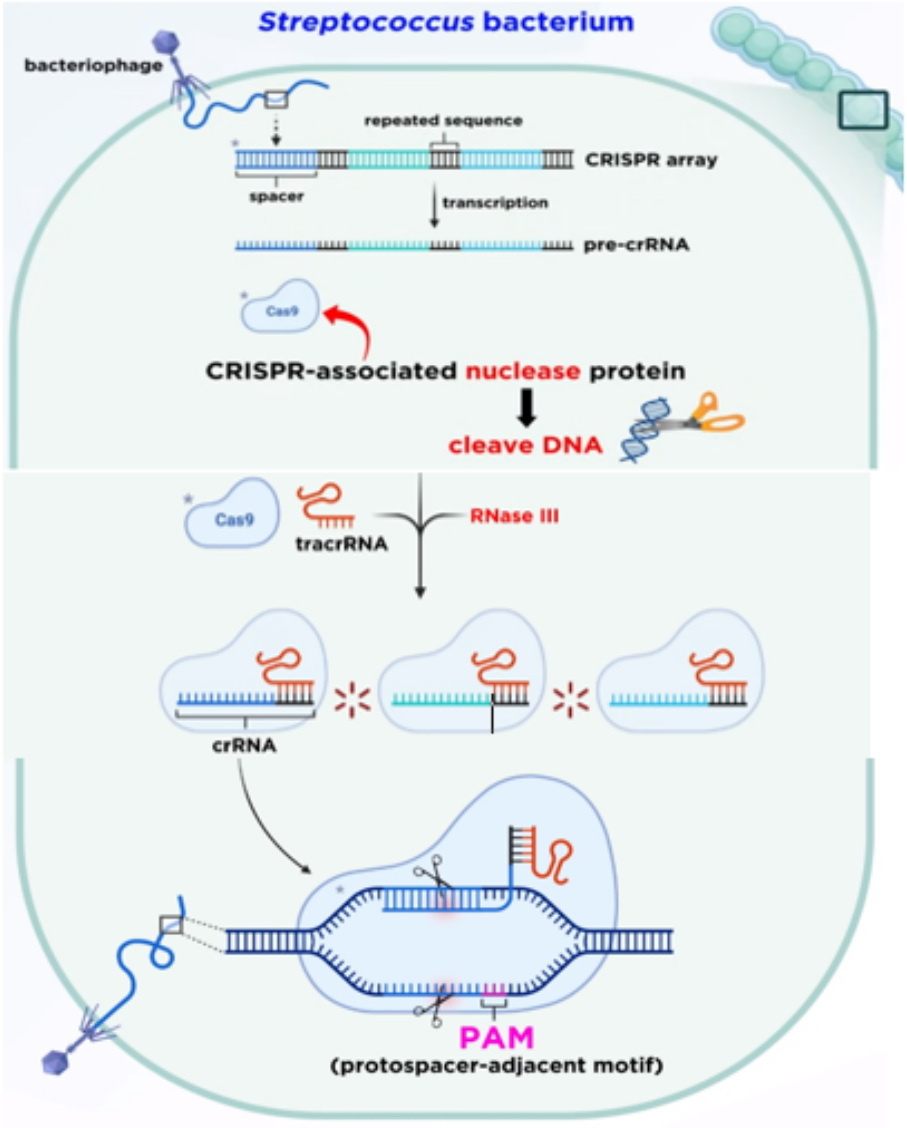

Image 2 Figure 6. Crispr cas 9 mechanism.

10.3. Restoration after cleavage

After cleavage, CRISPR-induced double-stranded DNA breaks can be repaired by either non-homologous end-joining (NHEJ) or homology-directed repair (HDR). NHEJ is the more frequently used, faster repair mechanism, because the cell does not use a template to join broken DNA ends together. It is, however, an error-prone process that can introduce mutations in the target sequence. Errors are rare, but when the break is repaired correctly,

Cas9 will once again recognize the target sequence and cleave it. Repeated cycles of cleavage and repair eventually result in a mutation. The type of mutation is random, but it will occur precisely within the desired target sequence. If the target sequence is within a gene's coding region, the mutation will likely inactivate that gene. The second type of repair mechanism is homology-directed repair (HDR), which is less error-prone and uses a homologous DNA template to accurately repair the break (for example, from a sister chromatid). Scientists can manipulate this repair system by introducing into the cell an excess of a DNA repair template along with the Cas9guide RNA complex. The cell's repair machinery will be "tricked" into using the repair template to fix the break by HDR. By designing different repair templates, scientists can change the target DNA sequence into a new sequence. These templates could also correct an existing mutation by replacing it with a non-mutated sequence of DNA.

(cf. CRISPR: Double-strand break and 2 types of editing)

11. Exercice : Which statement describes how CRISPR-Cas9 works?

[solution n°5 p. 19]

How CRISPR-Cas9 works?

- ☐ CRISPR-Cas9 uses guide RNA to target specific DNA sequences.
- ☐ CRISPR-Cas9 induces double-stranded breaks in the DNA at targeted locations.
- ☐ CRISPR-Cas9 modifies RNA sequences.
- ☐ CRISPR-Cas9 enhances gene expression without altering DNA sequences.

12. Selectable and screening markers

Selectable marker genes are a vital part of most transformation protocols. They are delivered alongside the gene of interest, either on the same plasmid or on a separate plasmid. As shown in table2, a wide range of selectable marker regimes is available and is particularly important in species where transformation efficiencies are low. Selectable marker genes can be categorized into those based on resistance genes that confer the ability to grow in the presence of toxic compounds such as antibiotics or herbicides which kill or otherwise compromise untransformed tissue (negative selection). Alternatively, a range of positive selection systems are available which provide transformed tissues with an enhanced ability to utilize, for example, an unusual carbohydrate or amino acid supply and thus enrich the culture for transformed tissue expressing the marker gene.

Host	Selection Markers
Prokaryotic cells	Ampicillin, Kanamycin, tetracycline, chloramphenicol
Yeast	HIS4, Zeocin, Auxotrophy
Mammalian cells	Neomycin, Puromycin, Hygromycin, Zeocin

Tableau 2 Table 2. Different selectable markers used in host.

12.1. The lac operon

The lac operon is an operon, or group of genes with a single promoter (transcribed as a single mRNA). The genes in the operon encode proteins that allow the bacteria to use lactose as an energy source. *E. coli* bacteria can break down lactose, but it's not their favorite fuel. If glucose is around, they would much rather use that. Glucose requires fewer steps and less energy to break down than lactose. However, if lactose is the only sugar available, the *E. coli* will go right ahead and use it as an energy source.

Définition **:**

The lac operon contains three genes: lacZ, lacY, and lacA. These genes are transcribed as a single mRNA, under control of one promoter. Genes in the lac operon specify proteins that help the cell utilize lactose. lacZ encodes an enzyme that splits lactose into monosaccharides (single-unit sugars) that can be fed into glycolysis. Similarly, lacY encodes a membrane-embedded transporter (permease) that helps bring lactose into the cell. lacA - Transacetylase encodes for Acetyltransferase that not directly involved in lactose metabolism or the regulation of the lac operon, lacA encodes for transacetylase, which acetylates some β-galactosides. In addition to the three genes, the *lac* operon also contains a number of regulatory DNA sequences *[7]*[7]*.

a) Action mechanism of Operon Lac

(cf. The Lac operon mechanism and regulation of gene expression)

12.2. The tryptophan operon

Bacteria such as *Escherichia coli* (a friendly inhabitant of our gut) need amino acids to survive because, like us, they need to build proteins. One of the amino acids they need is tryptophan. The trp operon includes five genes that encode enzymes needed for tryptophan biosynthesis, along with a promoter (RNA polymerase binding site) and an operator (binding site for a repressor protein). The genes of the trp operon are transcribed as a single mRNA.

Tryptophan operon (cf. 202004181556495169a89378.pdf)

a) Action mechanism of Operon Lac

(cf. Action mechanism of Tryptophan operon)

Conclusion

The chapter on expression vectors provides a thorough exploration of these fundamental tools in molecular biology and biotechnology. Expression vectors serve as essential vehicles for introducing and expressing foreign genes or genetic elements in host organisms, enabling researchers to manipulate gene expression, produce recombinant proteins, and study gene function. Throughout the chapter, various aspects of expression vectors have been discussed, including their structure, design principles, features, and applications.

One of the key highlights of the chapter is the diversity of expression vector systems available for different organisms and experimental requirements. From bacterial, yeast, and mammalian expression systems to viral vectors and plant transformation vectors, researchers have access to a wide range of expression platforms tailored to specific host organisms and applications. This diversity allows for the expression of genes in a variety of cellular environments, from simple prokaryotic systems to complex eukaryotic systems, each with its own advantages and limitations.

The chapter also emphasizes the importance of vector design in optimizing gene expression and protein production. Factors such as promoter strength, transcriptional regulatory elements, selection markers, and fusion tags play critical roles in modulating gene expression levels, protein solubility, and post-translational modifications. Rational vector design strategies, combined with advances in synthetic biology and genetic engineering, continue to enhance the efficiency and versatility of expression vector systems for diverse applications.

Furthermore, the chapter discusses practical considerations and techniques for vector construction, manipulation, and analysis. Methods for cloning, transformation, screening, and validation of recombinant vectors are essential skills for molecular biologists working with expression systems. Additionally, the chapter covers advanced topics such as vector

optimization, codon optimization, vector stability, and vector-host interactions, providing insights into troubleshooting common challenges encountered in vector-based experiments.

In terms of applications, expression vectors have revolutionized many areas of biological research, biotechnology, and medicine. From basic research applications, such as gene function studies, protein expression, and metabolic engineering, to applied applications in biopharmaceutical production, gene therapy, and vaccine development, expression vectors are indispensable tools driving scientific discovery and technological innovation.

In summary, the chapter on expression vectors underscores their pivotal role in molecular biology research and biotechnological applications. As our understanding of gene expression and genetic manipulation continues to advance, expression vectors will remain essential tools for unraveling the complexities of biological systems, engineering novel biomolecules, and addressing global challenges in health, agriculture, and environmental sustainability.

II Practices exercises

Définition **: Exercise about all the expression vector chapter**

Practical exercises on expression vectors involve hands-on activities aimed at understanding the principles and techniques associated with the use of expression vectors in molecular biology.

Practices exercises for chapter 01 (cf. TD01.pdf)

III Exam on chapter 1

Exercice 1 : Screening of the host cell

[solution n°6 p. 19]

Which of the following statements about DNA isolation from *E. coli* is not correct?

- O Chemical extraction using phenol removes proteins from the DNA.
- O RNA is removed from the sample by RNase treatment.
- O Detergent is used to break apart plant cells to extract DNA.
- O Centrifugation separates cellular components based on size.

Exercice 2 : Melting point of DNA

[solution n°7 p. 19]

Why does the GC content of a particular DNA molecule affect the melting of the two strands?

- O The G and C bond only requires two hydrogen bonds, thus requiring a lower temperature to “melt” the DNA.
- O Because G and C base-pairing requires three hydrogen bonds and a higher temperature is required to “melt” the DNA.
- O The nucleotide content of a DNA molecule is not important to know for biotechnology and molecular biology research.
- O None of the above.

Exercice 3 : Do you know promoter?

[solution n°8 p. 19]

What is the function of a promoter sequence in an expression vector?

- ☐ To terminate gene expression.
- ☐ To initiate transcription of the inserted gene.
- ☐ To regulate the vector's antibiotic resistance.
- ☐ To control gene expression.

Exercice 4 : Some of the steps of DNA isolation are given below.

[solution n°9 p. 20]

Reorder the steps

1. Transforming
2. Selecting
3. Cutting
4. Ligation
5. Cloning
6. Identification & Isolation

Réponse : ___ ___ ___ ___ ___ ___

Exercice 5 : Knowledge about biotechnology

[solution n°10 p. 20]

What is the primary purpose of using recombinant vectors in biotechnology?

Exercice 6 : Inducible promoter

[solution n°11 p. 20]

What is the significance of using inducible promoters in recombinant vectors?

Exercice 7 : Size of yeast vector

[solution n°12 p. 20]

From what you have learnt, can you tell what is the size of pBR322?

Exercice 8 : Size of vector

[solution n°13 p. 20]

From what you have learnt, can you tell what is the size of YAC?

Exercice 9 : Size of expression vector

[solution n°14 p. 20]

From what you have learnt, can you tell what is the size of cosmids?

Exercice 10 : Human genome size

[solution n°15 p. 20]

As a molecular biologist, what is the molar concentration of human DNA in a human cell?

IV ReOrientation Strategy

Strengthen their understanding

(cf. Expression vectors: how to choose, or customize, vectors for gene & protein expression)

Solutions des exercices

Solution n°1

[exercice p. 7]

Plasmids elimination?

- ○ By using antibiotics
- ○ By exposing them to optimal temperatures
- ○ By a process involving restriction enzymes and ligase
- ◉ By letting them undergo spontaneous elimination

Solution n°2

[exercice p. 7]

Yeast expression vector like plasmids?

- ○ YEps
- ○ YIps
- ○ YACs
- ◉ YRps

Solution n°3

[exercice p. 8]

Transformation

- ◉ Lentivirus
- ○ Ultrasound mediated gene transfer
- ○ Lithium acetate
- ○ Adenovirus

Solution n°4

[exercice p. 8]

The method that involves the introduction of purified phage DNA molecules into a `bacterial` cell is called `transfection`.

Solution n°5

[exercice p. 11]

How CRISPR-Cas9 works?

- ☑ CRISPR-Cas9 uses guide RNA to target specific DNA sequences.
- ☑ CRISPR-Cas9 induces double-stranded breaks in the DNA at targeted locations.
- ☐ CRISPR-Cas9 modifies RNA sequences.
- ☐ CRISPR-Cas9 enhances gene expression without altering DNA sequences.

Solution n°6

[exercice p. 15]

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- ○ RNA is removed from the sample by RNase treatment.
- ◉ Detergent is used to break apart plant cells to extract DNA.
- ○ Centrifugation separates cellular components based on size.

Solution n°7

[exercice p. 15]

Why does the GC content of a particular DNA molecule affect the melting of the two strands?

- ○ The G and C bond only requires two hydrogen bonds, thus requiring a lower temperature to “melt” the DNA.
- ◉ Because G and C base-pairing requires three hydrogen bonds and a higher temperature is required to “melt” the DNA.
- ○ The nucleotide content of a DNA molecule is not important to know for biotechnology and molecular biology research.
- ○ None of the above.

Solution n°8

[exercice p. 15]

What is the function of a promoter sequence in an expression vector?

- ☐ To terminate gene expression.
- ☑ To initiate transcription of the inserted gene.
- ☐ To regulate the vector's antibiotic resistance.
- ☑ To control gene expression.

Solution n°9

[exercice p. 15]

Reorder the steps

Cutting | Selecting | Ligation | Identification & Isolation | Cloning | Transforming

Different steps of DNA recombinant approach

Solution n°10

[exercice p. 16]

What is the primary purpose of using recombinant vectors in biotechnology?

Recombinant vectors are used in biotechnology to express and produce specific proteins, enzymes, or other molecules of interest.

Solution n°11

[exercice p. 16]

What is the significance of using inducible promoters in recombinant vectors?

Inducible promoters in recombinant vectors allow researchers to control gene expression by inducing it under specific conditions.

Solution n°12

[exercice p. 16]

From what you have learnt, can you tell what is the size of pBR322?

Few kilobases.

Solution n°13

[exercice p. 16]

From what you have learnt, can you tell what is the size of YAC?

Few kilobases to several tens of kilobases

Solution n°14

[exercice p. 16]

From what you have learnt, can you tell what is the size of cosmids?

30-50 kilobases

Solution n°15

[exercice p. 16]

As a molecular biologist, what is the molar concentration of human DNA in a human cell?

2 mg/ml of cell extract

Glossaire

Polymerase chain reaction (PCR)

A method widely used to make millions to billions of copies of a specific DNA sample rapidly, allowing scientists to amplify a very small sample of DNA (or a part of it) sufficiently to enable detailed study.

Abréviations

DNA : Deoxyribonucleic acid ; is the molecule that carries genetic information for the development and functioning of an organism.

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CRISPR: Double-strand break and 2 types of editing p. 11
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Table 2. Different selectable markers used in host. p. 11
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The Lac operon mechanism and regulation of gene expression p. 12
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Action mechanism of Tryptophan operon p. 12
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