Biotechnological Applications of Recombinant DNA

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Chapter II: Production of Fusion Proteins

1. Introduction

What is a fusion protein ?

A fusion protein is a protein made by rDNA technology that consists of at least two domains that are encoded by separate genes that have been joined so that they are transcribed and translated as a single unit, producing a single polypeptide. Fusion proteins can be created in vivo, for example, as the result of a chromosomal rearrangement. Fusion proteins can also be created in vitro using recombinant DNA techniques. The fusion often consists of a protein that is being studied joined to one of a small number of proteins that have useful properties to aid in the study.

2. Objectives

The chapter will permit to :

- Explore the concepts of fusion proteins and protein tagging techniques to gain a comprehensive understanding.
- Identify different types of protein tags.
- Understand the use of reporter genes to monitor gene expression patterns and regulatory mechanisms.
- Gain insights into the optimization of enzymatic cleavage conditions and protein solubilization strategies.

3. Definition of tag protein

The production of recombinant proteins in a highly purified and well-characterized form has become a major task for the protein chemist working in the pharmaceutical industry. In recent years, several epitope peptides and proteins have been developed to over-produce recombinant proteins. These affinity-tag systems share the following features: (a) one-step adsorption purification; (b) a minimal effect on tertiary structure and biological activity; (c) easy and specific removal to produce the native protein; (d) simple and accurate assay of the recombinant protein during purification; (e) applicability to a number of different proteins. Nevertheless, each affinity tag is purified under its specific buffer conditions, which could affect the protein of interest.

Thus, several different strategies have been developed to produce recombinant proteins on a large scale. One approach is to use a very small peptide tag that should not interfere with the fused protein. The most commonly used small peptide tags are poly-Arg-, FLAG-, poly-His-, c-myc-, S-, and Strep II-tag. For some applications, small tags may not need to be removed. The tags are not as immunogenic as large tags and can often be used directly as an antigen in antibody production. The effect on tertiary structure and biological activity of fusion proteins with small tags depends on the location and on the amino acids composition of the tag. Another approach is to use large peptides or proteins as the fusion partner. The use of a large partner can increase the solubility of the target protein. The disadvantage is that the tag must be removed for several applications e.g. crystallization or

antibody production. In general, it is difficult to decide on the best fusion system for a specific protein of interest. This depends on the target protein itself (e.g. stability, hydrophobicity), the expression system, and the application of the purified protein *[8]*[8]*.

- Translation **fusion of sequences** coding a **recombinant protein** and a) **short peptides**[ex. (His)n, (Asp)n, (Arg)n...]. b) **protein domains, entire proteins**[ex. MBP, GST, thioredoxin ...].

- Engineering a tagged protein requires adding the **DNA encoding the tag** to either the **5' or 3' end** of the gene encoding the protein of interest to generate a single, **recombinant protein with a tag at the N-or C-terminus**. The stretch of amino acids containing a target **cleavage sequence (CS)** is included to **allow selective removal of the tag** (Figure 7).

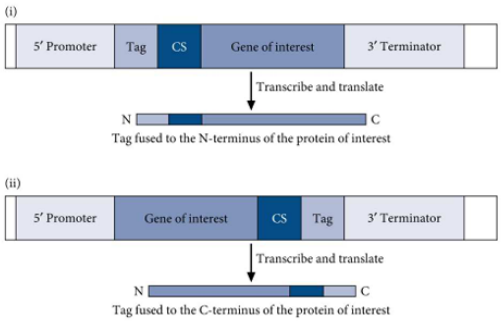

Figure 7. Tagged protein sites

What is a Protein Tag?

- Protein tag refers to the sub-domain or the peptide sequence of a fusion protein or molecules that are attached to a protein to modify its properties or function.
- Many kinds of protein tags available for recombinant protein production that can be used to identify, purify, or track proteins, and they have numerous applications in protein research and biotechnology.

(cf. Protein tagging)

Remarque **:**

Almost all recombinant proteins are prepared using fusion domains, also known as "tags" or protein tags.

4. Types of Tag

There are several types of protein tags commonly used in molecular biology and protein research. These tags are attached to the target protein of interest to facilitate purification, detection, localization, and/or functional studies. Some of the commonly used protein tags include *[9]*[9]*:

Types of tag protein (cf. euj72wc9a8l23dtu.pdf)

4.1. Polyarginine-tag (Arg-tag)

The Arg-tag was first described in 1984 and usually consists of five or six arginines. It has been successfully applied as C-terminal tag in bacteria, resulting in recombinant protein with up to 95% purity and a 44% yield. Arginine is the most basic amino acid. Arg5-tagged proteins can be purified by cation exchange resin SP-Sephadex, and most of the contaminating proteins do not bind. After binding, the tagged proteins are eluted with a linear NaCl gradient at alkaline pH. Polyarginine might affect the tertiary structure of proteins whose C-terminal region is hydrophobic. The Arg-tagged maltodextrin- binding protein of Pyrococcus furiosus has been crystalized. This enzymatic process has been successfully used in several instances, but often has been limited by poor cleavage yields or by unwanted cleavage occurred within the desired protein sequence. The Arg-tag can be used to immobilize functional proteins on flat surfaces; this is important for studying interactions with ligands. The Arg-tag is not used very often, in combination with a second tag it can be an interesting tool for protein purification.

4.2. Polyhistidine-tag(His-tag)

A widely employed method utilizes immobilized metal- affinity chromatography to purify recombinant proteins containing a short affinity-tag consisting of polyhistidine residues. Immobilized metal-affinity chromatography. Histidine is the amino acid that exhibits the strongest interaction with immobilized metal ion matrices, as electron donor groups on the histidine imidazole ring readily form coordination bonds with the immobilized transition metal. Peptides containing sequences of consecutive histidine residues are efficiently retained on IMAC. Following washing of the matrix material, peptides containing polyhistidine sequences can be easily eluted by either adjusting the pH of the column buffer or by adding free imidazole.

4.3. FLAG-tag

The FLAG-tag system utilizes a short, hydrophilic 8- amino-acid peptide that is fused to the protein of interest. The FLAG-tag can be located at the C- or N-terminus of the protein. The purification condition of the system is non-denaturing and thus allows active fusion proteins to be purified. The complex can be dissociated by chelating agents such as EDTA or by transiently reducing the pH.

4.4. Strep-tag

The Strep-tag is an amino acid peptide that was developed as an affinity tool for the purification of corresponding fusion proteins on streptavidin columns. Strep-tagged proteins are bound under physiological buffer conditions in the biotin binding pocket, and can be eluted gently with biotin derivatives. The tag can be engineered to either the C- or N-terminus of a protein. Recombinant Strep-tag-hybrids are produced in bacteria. The Strep-tag system is of relevance for studies on protein-protein interaction and special applications in which large or charged tags are not functional.

4.5. S-tag

The S-tag sequence is a fusion-peptide tag that allows detection by a rapid, sensitive homogeneous assay or by colorimetric detection in Western blots. The system is based on the strong interaction between the 15-amino- acid S-tag and the 103-amino-acid S-protein, both of which are derived from RNaseA. The tag is composed of four cationic, three anionic, three uncharged polar, and five non-polar residues. This composition makes the S-tag soluble. The S-tag rapid assay is based on the reconstitution of ribonucleolytic activity. Tagged proteins can be bound on S-protein matrices. The elution conditions are very harsh, e.g. buffer with pH 2. However, it is recommended to cleave the tag with protease to get functional proteins. The system is functional to purify recombinant proteins from bacteria.

4.6. Cellulose-binding domain

More than 13 different families of proteins with cellulose- binding domains (CBDs) have been classified. CBDs can vary in size from 4 to 20 kDa; they occur at different positions within polypeptides: N-terminal, C-terminal and internal. Some CBDs bind irreversibly to cellulose and can be used for immobilization of active enzymes. Hydrogen bond formation and van der Waals interaction are the main driving forces for binding. The advantage of cellulose is that it is inert, has low non-specific affinity, is available in many different forms, and has been approved for many pharmaceutical and human uses. CBDs bind to cellulose at a moderately wide pH range, from 3.5 to 9.5. The tag can be placed at the N- or C-terminus of the target protein. The affinity of the tag is so strong that an immobilized fusion protein can only be released with buffers containing urea or guanidine hydrochloride. This denaturating elution conditions make refolding of the fused target

protein necessary. Fused proteins with CBDs can be eluted gently from cellulose with ethylene glycol. This low-polarity solvent presumably disrupts the hydrophobic interaction at the binding site.

4.7. Maltose-binding protein

The 40-kDa maltose-binding protein (MBP) is encoded by the malE gene of *E. coli* K12. Vectors that facilitate the expression and purification of foreign peptides in *E. coli* by fusion to MPB were first described in 1988. Fused proteins can be purified by one-step affinity chromatography on cross-linked amylose. Bound fusion proteins can be eluted with 10 mM maltose in physiological buffer. Binding affinity is in the micro-molar range. The MBP-tag can be easily detected using an immuno- assay. It is necessary to cleave the tag with a site-specific protease. The MBP can be fused at the N- or C-terminus of the protein if the proteins are expressed in bacteria. N-terminal location can reduce the efficiency of translation. The MBP system is widely used in combination with a small affinity tag.

5. Exercice : NOT a feature of affinity-tag systems for recombinant proteins?

[solution n°1 p. 20]

The feature that is NOT commonly shared by affinity-tag systems for recombinant proteins is .

6. Exercice : Small peptide tags

[solution n°2 p. 20]

Small peptide tags may be preferred over peptides or as fusion partners due to their of .

7. Exercice : In the context of protein tagging, what is the purpose of including a stretch of amino acids containing a target cleavage sequence (CS)?

[solution n°3 p. 20]

Target cleavage sequence

- To increase the size of the fusion protein
- To prevent purification of the recombinant protein
- To allow selective removal of the tag
- To enhance protein synthesis

8. Uses of Protein Tags

Protein tags are widely used *in*[11]* molecular biology and protein research for various purposes *[10]*[10]*. Some common uses of protein tags include:

8.1. Purification of Recombinant Proteins

Purification of recombinant proteins is a crucial step in the production process to eliminate impurities and obtain a high-quality final product. Common methods of purification include chromatography, dialysis, centrifugation, and filtration. The choice of method depends on the nature of the protein, its impurities, and the desired purity level. In addition, it presents these properties:

- The recombinant protein to be produced is attached with a fusion tag.
- The tagged protein can be easily and conveniently purified by affinity chromatography.

Exemple **:**

Fusion tags available with affinity, such as glutathione S-transferase, ß-galactosidase, Maltose-binding protein, Polyhistidine tag, Cellulose-binding protein, and *Staphylococcus protein* A

8.2. Enhancing the Solubility of Recombinant Proteins

Enhancing the solubility of recombinant proteins is crucial for their successful expression, purification, and subsequent functional studies, as shown in figure 8 from [11].

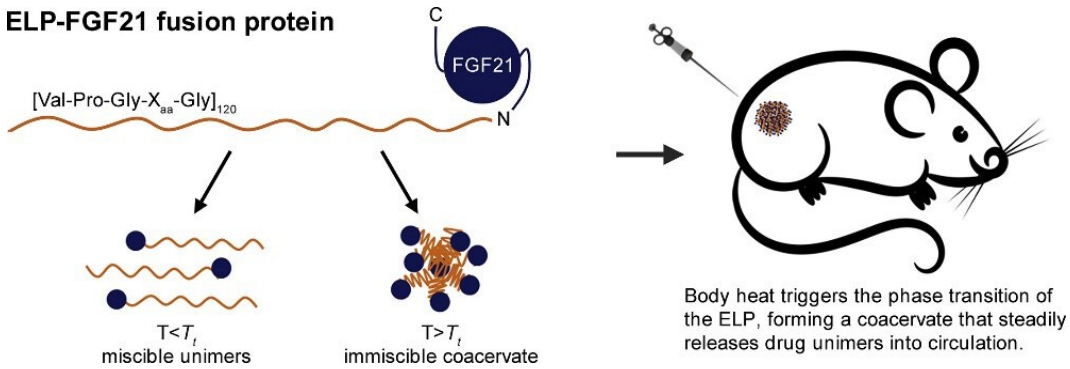

Image 1 Figure 8. Enhancing the Solubility of Recombinant Proteins.

a) Mechanism of action -Solubility-enhancing tags

- Maltose binding protein (MBP) might bind reversibly to exposed hydrophobic regions of nascent target polypeptide, steering the polypeptides towards their native conformation by a chaperone like –mechanism.
- NusA decreased translation rates by mediating transtriptional pausing, that might enable critical folding events to occur.
- Negative charged tags (highly acidic peptide) inhibit aggregation by increasing electrostatic repulsion between nascent polypepdides.

Exemple **: Example of Solubility-enhancing tags: *In vitro* using short peptide tags**

● Poly-Lys tag, poly-Arg tag= one, three and five lysine or arginine residues fused to the C- or N-terminus of the target protein.

● The solubilization effect of poly-Lys tags is lower than that of poly-Arg tags (lysines are less hydrophilic than arginines) of tagged BPTI-22 (bovine pancreatic trypsin inhibitor).

● The solubilization factor of all C-terminal tags was slightly higher than that of the respective N-terminal tags.

8.3. Membrane or Lipid-Binding Fusion Proteins

- These proteins facilitate the fusion of membranes or lipids, enabling the transport of molecules across cellular boundaries.
- Examples of membrane or lipid-binding fusion proteins include viral fusion proteins, which mediate viral entry into host cells, and membrane fusion proteins found in various neurological disorders.
- Understanding the structure and function of these proteins can provide insights into the development of therapeutic strategies for a range of diseases.

8.4. Toxin Fusion Proteins

Some fusion proteins include toxin fragments, allowing for targeted cell killing, such as immunotoxins used in cancer therapy. Interestingly, cost effective full-length IgG and IgG-fusion protein production in E. coli of made an attractive option for antibody production for research and hopefully for clinical applications.

8.5. Enzyme Fusion Proteins

Enzyme fusions, such as β-lactamase or alkaline phosphatase, are used for enzyme-linked assays or to monitor protein localization and activity.

8.6. Targeted Delivery Fusion Proteins

These fusions incorporate protein domains that specifically target them to particular cell types or subcellular compartments, improving precision in drug delivery and gene therapy.

8.7. Fusion Tags as reporters

Enable direct observation of dynamic intracellular processes. Reporter genes are used to observe expression of a specific gene, using GFP, luciferases, and Lac Z. To track the expression level of the specific protein. These tags are typically small and can be easily detected, purified, or localized within cells.

8.8. Combinatorial tagging

- No single tag is ideally suited for all purposes. Therefore, combinatorial tagging might be the only way to harness the full potential of tags in a high-throughput setting.
- Solubility-enhancing tag + purification tag: MBP + His6 tag
- 2xpurification tag:IgG-binding domain + streptavidin-binding domain
- Localization tag + purification tag: GFP + His6tag
- Localization tag + 2x purification tag + immunodetection: GFP + SBP domain + His8tag + c-Myc.

9. Reporter genes

Reporter genes are genes that are used in molecular biology and genetics to study the expression patterns, regulation, and localization of other genes or genetic elements. These genes encode proteins or enzymes that produce easily detectable signals, allowing researchers to track the activity of the reporter gene and indirectly monitor the activity of the target gene or genetic element. The video bellow explains the importance of these genes

(cf. Reporter genes and their importance)

9.1. Transcriptional fusion

Transcriptional fusion involves joining a promoter region (the regulatory sequence that controls gene expression) to a reporter gene (tag gene). It allows researchers to monitor the transcriptional activity of a gene by observing the expression of the reporter gene. This method helps in understanding when and where a gene is turned on (but not where the translated protein ends up). In a typical transcriptional fusion, the promoter region of a gene of interest is linked to a reporter gene, such as GFP (Green Fluorescent Protein). When the gene of interest is transcriptionally active, the reporter gene produces a visible signal (fluorescence in the case of GFP), indicating gene expression.

9.2. Translational Fusion

Translational fusion involves combining a GOI with a reporter gene in a way that their coding sequences are fused together, often resulting in a chimeric protein. It allows researchers to investigate the expression and localization of the chimeric protein, formed by the fusion of the target gene and the reporter gene. This method is used to study protein expression and function (and not necessarily where the RNA was transcribed). GOI is fused with a reporter gene, such as GFP, at the DNA level. The resulting protein, which contains a portion of the gene of interest and the reporter, is expressed and localized within the cell. By observing the fluorescence of GFP, researchers can track the protein's presence and movement.

10. Exercice : Which reporter gene is commonly used to indicate gene expression through visible fluorescence?

[solution n°4 p. 20]

reporter gene

- RFP
- GFP
- YFP
- CFP

11. Exercice : Reporter gene

[solution n°5 p. 20]

reporter gene

_____ fusion is a genetic technique that involves _____ a promoter region, which is the _____ sequence controlling gene expression, to a _____ gene.

12. Removal tags

Removal protein tags refers to the process of removing biological tags or epitopes from proteins. These tags are often added to proteins for identification or labelling purposes, but their presence can interfere with protein interaction studies or structure determination. There are several methods for removing protein tags, including enzymatic cleavage, chemical modification, or genetic engineering strategies. We can cite them as follow *[12]*[12]*:

12.1. Chemical cleavage

Exemple **:**

Chemical cleavage is a harsh method, efficient, but rather non-specific and may lead to unnecesary denaturation or modification of the target protein.

Cyanogen bromide (CNBr) cleaves at C- terminal of methionine (Met) residues

Hydroxylamine (NH2OH) cleaves at Asn-Gly (NG) sites in proteins

12.2. Self -cleaving

Self-cleavage is a process in which a protein releases itself from its binding site or frees itself from its active form after performing its function. This can be achieved through different mechanisms:

a) Intervening proteins

Inteins (intervening proteins) are protein segments that can excise themselves from protein precursors in which the are inserted and rejoin the flanking regions.

b) Self-Cleaving Peptide Sequences

System based on the catalytic domain of **Staphylococcus aureus sortase A**(SrtA). SrtA **cleaves** the **Thr-Gly** bond at the conserved **LPXTG** motif in the substrates. Cleavage is inducible by adding calcium (cofactor of Srt A).

c) FrpC modul

FrpC protein **undergoes calcium–inducible autocatalytic proccesing** at the peptide bond between residues Asp and Pro.

d) N-terminal protease (Npro)

The first protein of the pestivirus polyprotein. It posesses auto proteolytic activity and catalyzes the cleavage by switching from chaotropic to cosmotropic conditions.

e) Proteolytic cleavage

Exemple **:**

Vibrio cholerae secretes a large **multifunctional auto processing repeats-in-toxin** (MARTX) toxin that under goes **proteolytic cleavage** during translocation into host cells. Proteolysis of the toxin is mediated by a conserved internal cystein protease domain (CPD), which is activated upon binding of inositol hexakisphosphate.

12.3. Enzymatic cleavage

- **Unspecific cleavage** (optimization of protein cleavage conditions or using re-engineered proteases with increased specificity such as ProTEV and AcTEV proteases).
- **Optimization of protein cleavage conditions** (mainly enzyme-to-substrate ratio, temperature, pH, salt concentration, length of exposure).
- **Precipitation of the target protein when the fusion partner is removed**(approach for protein sollubilization has to be found, figure 9)

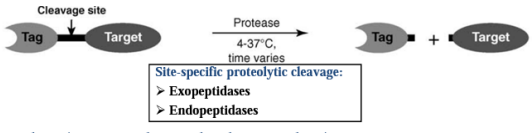

Figure 9. Enzymatic cleavage.

13. Exercice : What is the catalytic domain of Staphylococcus aureus sortase A (SrtA) known to cleave at?

[solution n°6 p. 21]

removal tags

- ☐ Thr-Gly bond
- ☐ Arg-Lys bond
- ☐ Asp-Pro bond
- ☐ LPXTG motif

14. Exercice : Difference between removal tags approaches

[solution n°7 p. 21]

Enzymatic cleavage | Chemical cleaving | Self -cleaving | Self -cleaving

Inteins	AcTEV proteases	N-terminal protease	Hydroxylamine

15. Exercice : Which chemical cleavage agent cleaves at the C-terminal of methionine (Met) residues?

[solution n°8 p. 21]

Chemical cleaving

- Cyanogen bromide (CNBr)
- Hydroxylamine (NH2OH)
- Trypsin
- Chymotrypsin

16. Purification

Table 3 presents the summary of two most used methods to purify the fusion protein

Characteristics	Pull-Down Assay	Co-Immunoprecipitation (Co-IP)
Objective	To isolate and identify proteins that interact with a specific bait protein.	To isolate and identify proteins that interact with a specific bait protein.
Bait Protein	The bait protein is immobilized onto a solid support (e.g., beads or resin).	Typically, the bait protein is in its native form within the cellular context.
Support Material	Solid support (e.g., beads or resin) with immobilized bait protein.	Antibodies specific to the bait protein or an associated tag.
Protein of Interest (POI)	Bait protein is the primary focus, and interactors are pulled down with it	Bait protein, along with any interaction partners (co-immunoprecipitated).
Immobilization Method	Bait protein is typically tagged and immobilized using affinity tags or antibodies.	Antibodies specific to the bait protein are used to capture the bait and its interactors.
Sample Complexity	Often used with purified samples or overexpressed proteins for controlled conditions.	Often used with purified samples or overexpressed proteins for controlled conditions.
Detection Method	Relies on the affinity of the immobilized bait protein for its interacting partners.	Proteins are analyzed by Western blotting or mass spectrometry, often using antibodies specific to the t
Specificity	Relies on the affinity of the immobilized bait protein for its interacting partners.	Relies on the affinity of the immobilized bait protein for its interacting partners.
In *Vivo* vs. In *Vitro*	Can be used in both in vitro (purified proteins) and in vivo (cellular context) settings.	Typically performed in the in vivo context of cell lysates or tissues.
Applications	Primarily used for the identification of proteins that interact with a specific bait protein.	Used to study protein-protein interactions and protein complexes within cells.

Tableau 1 Table 3. Difference between co-immunoprecipitation and pull-down assay in protein fusion purifcation

17. Exercice : Pull down assay and Co-Immunoprecipitation differences

[solution n°9 p. 21]

Pull-Down Assay **Co-Immunoprecipitation** **Co-Immunoprecipitation**

Pull-Down Assay **Pull-Down Assay** Co-Immunoprecipitation

Co-Immunoprecipitation

isolate and identify proteins that interact with a specific bait protein.	Typically, the bait protein is in its native form within the cellular context.	Bait protein, along with any interaction partners	Antibodies specific to the bait protein are used to capture the bait and its interactors.	Proteins are eluted and detected using methods like gel electrophoresis, Western blotting, mass spectrometry, or enzymatic assays.	Typically performed in the in vivo context of cell lysates or tissues	Relies on the affinity of the immobilized bait protein for its interacting partners

Conclusion

In conclusion, the chapter on fusion proteins provides a comprehensive overview of this important concept in molecular biology and biotechnology. Fusion proteins, created by the fusion of two or more protein domains or sequences, offer a wide range of applications in research, medicine, and industry. Throughout the chapter, various aspects of fusion proteins have been discussed, including their structure, design strategies, production methods, and applications.

One of the key advantages of fusion proteins is their versatility in addressing diverse research questions and technological challenges. By combining different protein domains or functional motifs, fusion proteins can be tailored to perform specific functions or exhibit desired properties. This flexibility enables researchers to develop novel tools and techniques for studying biological processes, engineering proteins with improved properties, and designing therapeutic agents for treating diseases.

The chapter also highlights the importance of rational design and optimization strategies in the development of fusion proteins. Through rational design approaches, researchers can strategically select and combine protein domains to achieve desired outcomes, such as enhanced stability, solubility, specificity, or activity. Optimization of fusion protein expression systems, purification protocols, and characterization methods further enhances the utility and effectiveness of these molecules in various applications.

Moreover, the chapter discusses the diverse applications of fusion proteins across different fields. From basic research applications, such as protein localization, interaction studies, and signal transduction analysis, to practical applications in biotechnology, diagnostics, and therapeutics, fusion proteins play a crucial role in advancing scientific knowledge and technological innovation. Examples of specific applications include the development of biosensors, drug delivery systems, affinity purification tags, and therapeutic proteins.

In summary, the chapter on fusion proteins underscores the significance of this versatile tool in molecular biology and biotechnology. By harnessing the power of fusion proteins, researchers can address complex biological questions, overcome technical challenges, and develop innovative solutions to benefit society. As our understanding of protein structure-function relationships continues to evolve, fusion proteins will undoubtedly remain a valuable resource for driving scientific discovery and technological advancement in the years to come.

II Practices exercises

Définition **:**

These exercises teach students about the importance of tag removal for downstream applications and the factors influencing tag cleavage efficiency.

Practices exercises for chapter 02 (cf. TD02.pdf)

III Exam on chapter 2

Exercice 1 : Gene of interest

[solution n°10 p. 22]

What kind of reporter would you use to determine the expression pattern of GOI?

- Transcriptional
- Translational
- Translational
- Transactional

Exercice 2 : Expression of GOI

[solution n°11 p. 22]

Why is it important to determine the expression pattern of GOI?

- To understand genetic mutations
- To identify molecular pathways
- To improve cellular communication
- To enhance protein stability

Exercice 3 : Transcription

[solution n°12 p. 22]

Which type of reporter directly reflects gene activity at the RNA level?

- Fluorescent protein
- Antibiotic marker
- DNA polymerase
- Reverse transcriptase

Exercice 4 : Reporter gene

[solution n°13 p. 22]

What is the main purpose of a reporter gene assay?

- To investigate the structure of a gene
- To identify the function of a gene
- To examine the promoter of a gene and its activity
- To manipulate gene expression

Exercice 5 : Reporter gene

[solution n°14 p. 23]

In the context of reporter gene assays, what does it mean if a promoter is considered "weak"?

- It leads to high gene expression
- It results in low gene expression
- It has no impact on gene expression
- It regulates gene transcription

Exercice 6 : Fusion protein

[solution n°15 p. 23]

What kind of reporter would you use to determine the subcellular location of a protein of interest and why?

Exercice 7 : Knowledge about self-processing

[solution n°16 p. 23]

What is a self-processing model?

Exercice 8 : Reporter gene

[solution n°17 p. 23]

What kind of reporter would you use to test protein mobility between cells and why?

IV ReOrientation Strategy

Enhance their comprehension

(cf. euj72wc9a8l23dtu.pdf)

Solutions des exercices

Solution n°1

[exercice p. 7]

The feature that is NOT commonly shared by affinity-tag systems for recombinant proteins is `immunogenicity`.

Solution n°2

[exercice p. 7]

Small peptide tags may be preferred over `large` peptides or `proteins` as fusion partners due to their `ease` of `removal`.

Solution n°3

[exercice p. 7]

Target cleavage sequence

- ○ To increase the size of the fusion protein
- ○ To prevent purification of the recombinant protein
- ◉ To allow selective removal of the tag
- ○ To enhance protein synthesis

Solution n°4

[exercice p. 10]

reporter gene

- ○ RFP
- ◉ GFP
- ○ YFP
- ○ CFP

Solution n°5

[exercice p. 10]

reporter gene

`Transcriptional` fusion is a genetic technique that involves `joining` a promoter region, which is the `regulatory` sequence controlling gene expression, to a `reporter` gene.

Solution n°6

[exercice p. 12]

removal tags

- [x] Thr-Gly bond
- [ ] Arg-Lys bond
- [ ] Asp-Pro bond
- [x] LPXTG motif

Solution n°7

[exercice p. 12]

Inteins	AcTEV proteases	N-terminal protease	Hydroxylamine
Self -cleaving	Enzymatic cleavage	Self -cleaving	Chemical cleaving

Solution n°8

[exercice p. 13]

Chemical cleaving

- (•) Cyanogen bromide (CNBr)
- ( ) Hydroxylamine (NH2OH)
- ( ) Trypsin
- ( ) Chymotrypsin

Solution n°9

[exercice p. 15]

cally, the bait n is in its native m within the ular context.	**Bait protein, along with any interaction partners**	**Antibodies specific to the bait protein are used to capture the bait and its interactors.**	**Proteins are eluted and detected using methods like gel electrophoresis, Western blotting, mass spectrometry, or enzymatic assays.**	**Typically perform in the in vivo cont of cell lysates o tissues**
Co-noprecipitation	**Co-Immunoprecipitation**	Co-Immunoprecipitation	Pull-Down Assay	Co-Immunoprecipitat

Solution n°10

[exercice p. 17]

What kind of reporter would you use to determine the expression pattern of GOI?

- ◉ Transcriptional
- ○ Translational
- ○ Translational
- ○ Transactional

Solution n°11

[exercice p. 17]

Why is it important to determine the expression pattern of GOI?

- ○ To understand genetic mutations
- ◉ To identify molecular pathways
- ○ To improve cellular communication
- ○ To enhance protein stability

Solution n°12

[exercice p. 17]

Which type of reporter directly reflects gene activity at the RNA level?

- ◉ Fluorescent protein
- ○ Antibiotic marker
- ○ DNA polymerase
- ○ Reverse transcriptase

Solution n°13

[exercice p. 17]

What is the main purpose of a reporter gene assay?

- ○ To investigate the structure of a gene
- ○ To identify the function of a gene
- ◉ To examine the promoter of a gene and its activity
- ○ To manipulate gene expression

Solution n°14

[exercice p. 18]

In the context of reporter gene assays, what does it mean if a promoter is considered "weak"?

- ○ It leads to high gene expression
- ◉ It results in low gene expression
- ○ It has no impact on gene expression
- ○ It regulates gene transcription

Solution n°15

[exercice p. 18]

What kind of reporter would you use to determine the subcellular location of a protein of interest and why?

The translational because the single fused protein of interest in GFP will enter the compartment of the protein of interest localization signal tells it to go.

Solution n°16

[exercice p. 18]

What is a self-processing model?

A structural element or peptide sequence within the fusion protein that possesses intrinsic enzymatic activity, allowing it to cleave or process itself without the need for external enzymes or co-factors.

Solution n°17

[exercice p. 18]

What kind of reporter would you use to test protein mobility between cells and why?

Transcriptional and translational because we have to know both where the GOI was transcribed and was translated product ends up to determine mobility.

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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