

# Biotechnological Applications of Recombinant DNA

Dr. Selma Hamimed

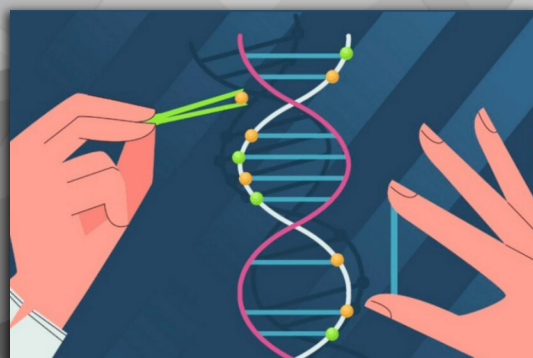
Department of Molecular and Cellular Biology

Faculty of Natural Sciences and Life

University of Jijel

Email: [selma.hamimed@univ-jijel.dz](mailto:selma.hamimed@univ-jijel.dz)<sup>1</sup>

1.0 November 2024



Dr. Selma Hamimed

<sup>1</sup> file:///C:/Users/Selma/Downloads/TiO2nanovitel-3/Cours%20%C3%A0%20reproduire%20version%20fran%C3%A7ais%20(1)/Cours%20%C3%A0%20reproduire\_gen\_2

# Table des matières

|                                |           |
|--------------------------------|-----------|
| <b>Objectifs</b>               | <b>3</b>  |
| <b>Introduction</b>            | <b>4</b>  |
| <b>I - Prerequisites</b>       | <b>6</b>  |
| <b>Solutions des exercices</b> | <b>8</b>  |
| <b>Glossaire</b>               | <b>10</b> |
| <b>Abréviations</b>            | <b>11</b> |
| <b>Bibliographie</b>           | <b>12</b> |

# Objectifs

Recombinant DNA technology has proven important in producing vaccines and protein therapies such as human insulin, interferon and human growth hormone. Overall, this course aims to highlight and provide students with a comprehensive understanding of the techniques, principles, and applications of recombinant DNA technology in genetic engineering to address societal needs, improve human health, enhance food and energy production, and promote sustainable development. By the end of the course, students will be able to:

- Explain the fundamental concepts of recombinant DNA technology, including gene cloning, DNA manipulation, and genetic engineering techniques.
- Understand the significance of recombinant DNA technology in various fields such as medicine, agriculture, industry, and environmental science.
- Analyze and interpret results data generated through recombinant DNA techniques, and critically evaluate the validity and significance of research findings.
- Apply knowledge of recombinant DNA technology to solve practical problems and design experiments in biotechnology research and development.
- Assess current advancements and emerging trends in biotechnological applications of recombinant DNA.
- Communicate effectively about recombinant DNA technology concepts, research findings, and applications through written reports, oral presentations, and scientific discussions.

# Introduction

What do you think of when you hear the word “**biotechnology**”? Maybe things you’ve seen in the news, such as Dolly the cloned sheep, genetically modified organisms, or gene therapy. If that’s what you think of, you’re absolutely right: these are all examples of biotechnology. But what about crop breeding and the antibiotic penicillin? These processes and products – some of which have been around for thousands of years – are also examples of biotechnology.

## What is biotechnology?

Biotechnology is the use of an organism, or a component of an organism or other biological system, to make a product or process for a specific use. This is a very broad definition, and as mentioned above, it can include both cutting-edge laboratory techniques and traditional agricultural and culinary techniques that have been practiced for hundreds of years [1]<sup>1</sup>\*. Let’s look at three examples of biotechnology and see how they fit the definition:

**Genetically Modified Crops (GMOs)**, also known as genetically engineered crops, are plants that have had their genetic material altered in a way that does not occur naturally through mating or natural recombination. This genetic modification is typically done in a laboratory to introduce specific traits or characteristics into the crops. These traits can be derived from other plants, animals, bacteria, or even synthetic sources.

**Penicillin:** The antibiotic penicillin is generated by certain molds. To make small amounts of penicillin for use in early clinical trials, researchers had to grow up to 500 liters of “mold juice” a week. The process has since been improved for industrial production, with use of higher-producing mold strains and better culture conditions to increase yield. Here, we see an organism (mold) being used to make a product for human use – in this case, an antibiotic to treat bacterial infections.

**Gene therapy:** Gene therapy is an emerging technique used to treat genetic disorders that are caused by a nonfunctional gene. It works by delivering the “missing” gene’s DNA to the cells of the body. For instance, in the genetic disorder cystic fibrosis, people lack function of a gene for a chloride channel produced in the lungs. In a recent gene therapy clinical trial, a copy of the functional gene was inserted into a circular DNA molecule called a plasmid and delivered to patients’ lung cells in spheres of membrane (in the form of a spray).

## What is DNA technology?

Many examples of modern biotechnology depend on the ability to analyze, manipulate, and cut and paste pieces of DNA\*. Approaches for the sequencing and manipulation of DNA are sometimes referred to as DNA technology. For example, for the cystic fibrosis gene therapy trial, researchers used DNA manipulation techniques to insert the chloride channel gene into a piece of carrier DNA (a vector) that allowed it to be expressed in human lung cells.

DNA technology is important to both basic and applied (practical) biology. For instance, a technique used to make many copies of a DNA sequence, called<sup>2</sup> *polymerase chain reaction*\* (PCR) [2]<sup>2</sup>\*, is used in many medical diagnostic tests and forensics applications as well as in basic laboratory research.

---

2. <https://www.khanacademy.org/science/biology/biotech-dna-technology/dna-sequencing-pcr-electrophoresis/a/polymerase-chain-reaction-pcr>

## What is recombinant DNA?

Recombinant DNA is a form of artificial DNA that is made through the combination or insertion of one or more DNA strands, therefore combining DNA sequences as per your requirement, within different species, as shown in the figure 1.

Recombinant DNA, also known as genetic engineering, is the process of combining DNA from different organisms to create a new and potentially useful organism [3]<sup>3\*</sup>. Here are the general steps for recombinant DNA in Figure 2:

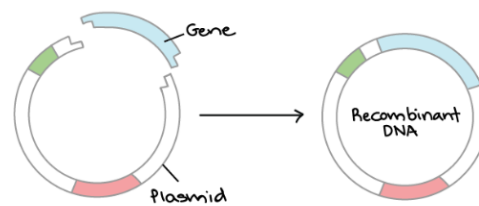


Figure 1. Illustrative image of Recombinant DNA technology

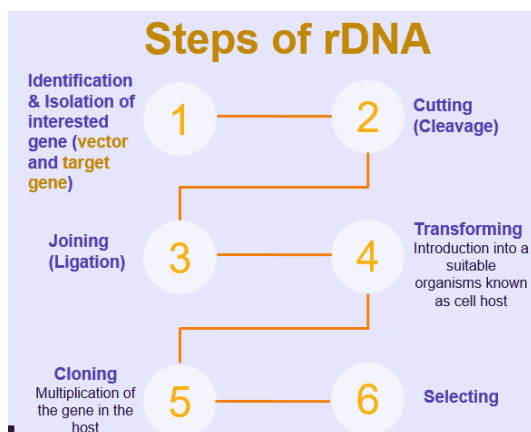
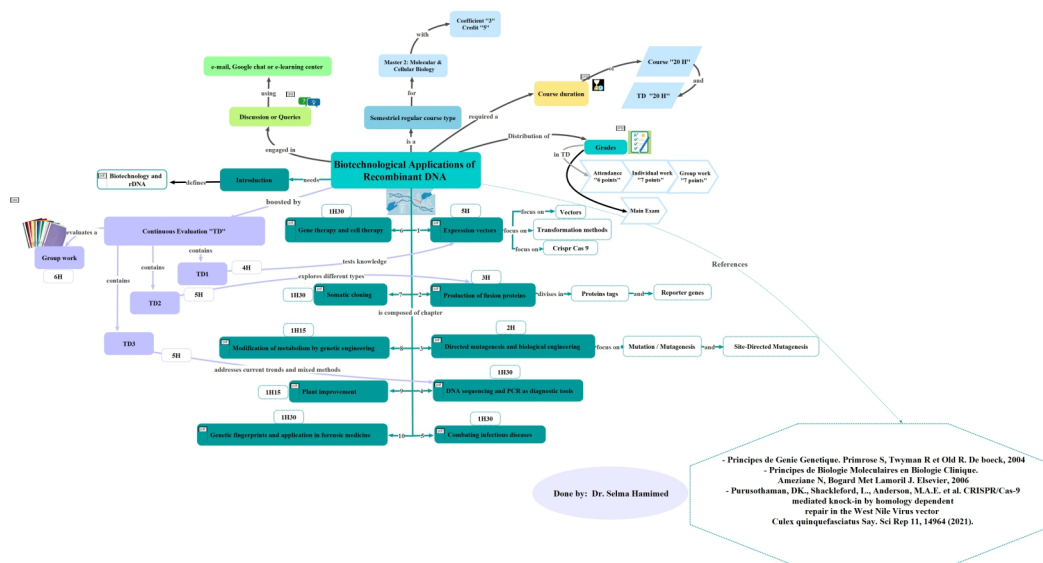


Figure 2. Overall steps for recombinant DNA

## Advantages of Recombinant technology:

- Provide substantial quantity;
- No need for natural or organic factors;
- Tailor made product that you can control;
- Unlimited utilizations;
- Cheap;
- Resistant to natural inhibitors.



Graphique 1 Figure 3. Mental map

# I Prerequisites

- Strong foundation in molecular genetics and genomics, including gene structure, regulation, and expression.
- Knowledge of genomic organization and analysis techniques is also essential.
- Understanding of biochemistry and cell biology: Familiarity with biochemical processes, cell structure, and cellular functions is crucial for comprehending the applications of recombinant DNA technology at the molecular and cellular levels.
- Knowing how to perform DNA extraction, gel electrophoresis, PCR, cloning, and bacterial transformation would help.
- Basic proficiency in data analysis and bioinformatics tools.

## Exercise 1 : How is gene structure defined?

[solution n°1 p. 8]

Gene structure characteristics?

- ☐ The arrangement of exons and introns within a gene
- ☐ The location of a gene on a chromosome
- ☐ The arrangement of nucleotides in a DNA sequence
- ☐ The number of alleles present in a gene

## Exercise 2 : What is the primary function of mRNA in gene expression?

[solution n°2 p. 8]

Transcription role?

- ☐ Carrying amino acids to the ribosome
- ☐ Acting as a template for DNA replication
- ☐ Transferring genetic information from the nucleus to the cytoplasm
- ☐ Initiating the translation process

## Exercise 3 : Which of the following is an example of post-transcriptional gene regulation?

[solution n°3 p. 8]

Examples demonstrate post-transcriptional gene regulation?

- ☐ Alternative splicing
- ☐ DNA methylation
- ☐ Acetylation
- ☐ mRNA degradation

## Exercice 4 : The regulation mechanism

[solution n°4 p. 8]

The lac operon is a  gene regulatory system that  the expression of genes involved in the  of .

## Exercice 5 : The steps in DNA extraction are:

[solution n°5 p. 9]

1.
2.
3.
4.
5.

Réponse :

# Solutions des exercices

## Solution n°1

[exercice p. 6]

Gene structure characteristics?

- ☒ The arrangement of exons and introns within a gene
- ☐ The location of a gene on a chromosome
- ☒ The arrangement of nucleotides in a DNA sequence
- ☐ The number of alleles present in a gene

## Solution n°2

[exercice p. 6]

Transcription role?

- ☐ Carrying amino acids to the ribosome
- ☐ Acting as a template for DNA replication
- ☒ Transferring genetic information from the nucleus to the cytoplasm
- ☐ Initiating the translation process

## Solution n°3

[exercice p. 6]

Examples demonstrate post-transcriptional gene regulation?

- ☒ Alternative splicing
- ☐ DNA methylation
- ☐ Acetylation
- ☒ mRNA degradation

## Solution n°4

[exercice p. 7]

The lac operon is a **bacterial** gene regulatory system that **controls** the expression of genes involved in the **metabolism** of **lactose**.



## Solution n°5

[exercice p. 7]

Precipitating DNA

Washing

Lysing cells

Purifying DNA

Pelleting

 The steps in DNA extraction

# 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.

# Bibliographie

- Gupta, V., Sengupta, M., Prakash, J., & Tripathy, B. C. (2016). An Introduction to Biotechnology. Basic and Applied Aspects of Biotechnology, 1–21. doi:10.1007/978-981-10-0875-7\_1
- Zhao, X., Li, G., & Liang, S. (2013). Several Affinity Tags Commonly Used in Chromatographic Purification. *Journal of Analytical Methods in Chemistry*, 2013, 1–8.
- Gilroy, C. A., Roberts, S., & Chilkoti, A. (2018). Fusion of fibroblast growth factor 21 to a thermally responsive biopolymer forms an injectable depot with sustained anti-diabetic action. *Journal of Controlled Release*, 277, 154–164. doi:10.1016/j.jconrel.2018.03.015
- Goh, H. C., Sobota, R. M., Ghadessy, F. J., & Nirantar, S. (2017). Going native: Complete removal of protein purification affinity tags by simple modification of existing tags and proteases. *Protein Expression and Purification*, 129, 18–24.
- Johnston, M. O. (2006). Mutations and New Variation: Overview. *Encyclopedia of Life Sciences*. doi:10.1038/npg.els.0004165
- Durland J, Ahmadian-Moghadam H. Genetics, Mutagenesis. [Updated 2022 Sep 19]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2024 Jan-. Available from: <https://www.ncbi.nlm.nih.gov/books/NBK560519/>
- Madhavan, A., Sindhu, R., Binod, P., Sukumaran, R. K., & Pandey, A. (2017). Strategies for design of improved biocatalysts for industrial applications. *Bioresource Technology*, 245, 1304–1313. doi:10.1016/j.biortech.2017.05
- Ling MM, Robinson BH. Approaches to DNA mutagenesis: an overview. *Anal Biochem*. 1997 Dec 15;254(2):157-78.
- Jabalameli HR, Zahednasab H, Karimi-Moghaddam A, Jabalameli MR. Zinc finger nuclease technology: advances and obstacles in modelling and treating genetic disorders. *Gene*. 2015 Mar 01;558(1):1-5.
- Shahzad, S., Afzal, M., Sikandar, S., & Afzal, I. (2020). Polymerase Chain Reaction. *Genetic Engineering - A Glimpse of Techniques and Applications*. doi:10.5772/intechopen.81924
- Dymond, J. S. (2013). Explanatory Chapter. *Laboratory Methods in Enzymology: DNA*, 279–289. doi:10.1016/b978-0-12-418687-3.00023-9
- Morrow, J. F. (1979). [1] Recombinant DNA techniques. *Recombinant DNA*, 3–24. doi:10.1016/0076-6879(79)68003-5
- Marcela AAV, Rafael LG, Lucas ACB, Paulo RE, Alessandra ATC, Sergio C. Principles and applications of polymerase chain reaction in medical diagnostic fields: A review. *Brazilian Journal of Microbiology*. 2009;40:1-11
- Joyce C. Quantitative RT-PCR. A review of current methodologies. *Methods in Molecular Biology*. 2002;193:83-92
- Rajeevan MS, Vernon SD, Taysavang N, Unger ER. Validation of array-based gene expression profiles by real-time (kinetic) RT-PCR. *The Journal of Molecular Diagnostics*. 2001;3(1):26-31
- Stephen B, Mueller R. Realtime reverse transcription PCR (qRT-PCR) and its potential use in clinical diagnosis. *Clinical Science*. 2005;109:365-379
- Lin MH, Chen TC, Kuo TT, Tseng C, Tseng CP. Real-time PCR for quantitative detection of *Toxoplasma gondii*. *Journal of Clinical Microbiology*. 2000;38:4121-4125

- Fortin NY, Mulchandani A, Chen W. Use of real time polymerase chain reaction and molecular beacons for the detection of *Escherichia coli* O157:H7. *Analytical Biochemistry*. 2001;289:281-288
- Jeyaseelan K, Ma D, Armugam A. Real-time detection of gene promotor activity: Quantification of toxin gene transcription. *Nucleic Acids Research*. 15 June 2001;29(12):e58
- Kadri, K. (2020). Polymerase Chain Reaction (PCR): Principle and Applications. *Synthetic Biology - New Interdisciplinary Science*. doi:10.5772/intechopen.86491
- Shehata HR, Hassane B and Newmaster SG (2024) Real-time PCR methods for identification and stability monitoring of *Bifidobacterium longum* subsp. *longum* UABI-14 during shelf life. *Front. Microbiol*. 15:1360241. doi: 10.3389/fmicb.2024.1360241
- Men, A. E., Wilson, P., Siemering, K., & Forrest, S. (2008). Sanger DNA Sequencing. *NextGeneration Genome Sequencing: Towards Personalized Medicine*, 1–11. <https://doi.org/10.1002/9783527625130.ch1> (PDF) SANGER`S DIDEOXY CHAIN TERMINATION METHOD OF DNA SEQUENCING. Available from: [https://www.researchgate.net/publication/378490879\\_SANGERS\\_DIDEOXY\\_CHAIN\\_TERMINATION\\_METHOD\\_OF\\_DNA\\_SEQUENCING](https://www.researchgate.net/publication/378490879_SANGERS_DIDEOXY_CHAIN_TERMINATION_METHOD_OF_DNA_SEQUENCING) [accessed Oct 16 2024].
- Wilson, A. J., Morgan, E. R., Booth, M., Norman, R., Perkins, S. E., Hauffe, H. C., ... Fenton, A. (2017). What is a vector? *Philosophical Transactions of the Royal Society B: Biological Sciences*, 372(1719), 20160085.
- Dunbar, C. E., High, K. A., Joung, J. K., Kohn, D. B., Ozawa, K., & Sadelain, M. (2018). Gene therapy comes of age. *Science*, 359(6372), eaan4672. doi:10.1126/science.aan4672
- Ledley, F.D. Pharmaceutical Approach to Somatic Gene Therapy. *Pharm Res* 13, 1595–1614 (1996). <https://doi.org/10.1023/A:1016420102549>
- Munung, N.S., Nnodu, O.E., Moru, P.O. et al. Looking ahead: ethical and social challenges of somatic gene therapy for sickle cell disease in Africa. *Gene Ther* 31, 202–208 (2024). <https://doi.org/10.1038/s41434-023-00429-7>
- Wolf, D.P., Mitalipov, P.A. & Mitalipov, S.M. Principles of and strategies for germline gene therapy. *Nat Med* 25, 890–897 (2019). <https://doi.org/10.1038/s41591-019-0473-8>
- Nishikawa, M., & Hashida, M. (2002). Nonviral Approaches Satisfying Various Requirements for Effective in Vivo Gene Therapy. *Biological & Pharmaceutical Bulletin*, 25(3), 275–283. doi:10.1248/bpb.25.275
- Gowing, G., Svendsen, S., & Svendsen, C. N. (2017). Ex vivo gene therapy for the treatment of neurological disorders. *Progress in Brain Research*, 99–132. doi:10.1016/bs.pbr.2016.11.003
- Wivel, N. A., & Wilson, J. M. (1998). METHODS OF GENE DELIVERY. *Hematology/Oncology Clinics of North America*, 12(3), 483–501. doi:10.1016/s0889-8588(05)70004-6
- Wang D, Gao G. State-of-the-art human gene therapy: part II. Gene therapy strategies and clinical applications. *Discov Med*. 2014 Sep;18(98):151-61. PMID: 25227756; PMCID: PMC4440458.
- El-Kadiry AE-H, Rafei M and Shammaa R (2021) Cell Therapy: Types, Regulation, and Clinical Benefits. *Front. Med*. 8:756029.
- Fléchon, J. E., Kopečný, V., Pivko, J., Pavlok, A., & Motlik, J. (2004). Texture of the zona pellucida of the mature pig oocyte. The mammalian egg envelope revisited. *Reproduction Nutrition Development*, 44(3), 207–218. doi:10.1051/rnd:2004026
- Bertero, A., Brown, S., & Vallier, L. (2017). Methods of Cloning. *Basic Science Methods for Clinical Researchers*, 19–39. doi:10.1016/b978-0-12-803077-6.00002-3 10.1016/B978-0-12-8030
- Tian, X.C., Kubota, C., Enright, B. et al. Cloning animals by somatic cell nuclear transfer – biological factors. *Reprod Biol Endocrinol* 1, 98 (2003). <https://doi.org/10.1186/1477-7827-1-98>

- Wells DN, Laible G, Tucker FC, Miller AL, Oliver JE, Xiang T, Forsyth JT, Berg MC, Cockrem K, L'Huillier PJ, Tervit HR, Obach B: Coordination between donor cell type and cell cycle stage improves nuclear cloning efficiency in cattle. *Theriogenology*. 2003, 59: 45-59. 10.1016/S0093-691X(02)01273-6.
- Enright, B. P., Kubota, C., Yang, X., & Tian, X. C. (2003). Epigenetic Characteristics and Development of Embryos Cloned from Donor Cells Treated by Trichostatin A or 5-aza-2'-deoxycytidine. *Biology of Reproduction*, 69(3), 896–901. doi:10.1095/biolreprod.103.017954
- Samiec M. Molecular Mechanism and Application of Somatic Cell Cloning in Mammals-Past, Present and Future. *Int J Mol Sci*. 2022 Nov 9;23(22):13786. doi: 10.3390/ijms232213786. PMID: 36430264; PMCID: PMC9697074.
- Benedito VA, Modolo LV. Introduction to metabolic genetic engineering for the production of valuable secondary metabolites in in vivo and in vitro plant systems. *Recent Pat Biotechnol*. 2014;8(1):61-75. doi: 10.2174/1872208307666131218125801. PMID: 24354528.
- Lessard, P. (1996). Metabolic engineering, the concept coalesces. *Nature Biotechnology* 14: 1654-1655.
- Stephanopoulos, G. and Vallino, J.J. (1991). Network rigidity and metabolic engineering in metabolite overproduction. *Science* 252:1675-1681.
- Liao, J.C., Hou, S.Y. and Chao, Y.P. (1996). Pathway analysis, engineering, and physiological considerations for redirecting central metabolism. *Biotechnology & Bioengineering* 52:129-140.
- Shimada, H., Kondo, K., Fraser, P. D., Miura, Y., Saito, T and, Misawa, N. (1998). Increased carotenoid production by the food yeast *Candida utilis* through metabolic engineering of the isoprenoid pathway. *Applied Microbiology and Biotechnology* 64:2676-2680.
- Smirnoff, N. (1998). Plant resistance to environmental stress. *Current Opinion in Biotechnology* 9:214-219.
- Chiew, K. L., Yong, K. S. C., & Tan, C. L. (2018). A survey of phishing attacks: Their types, vectors and technical approaches. *Expert Systems with Applications*, 106, 1–20. doi:10.1016/j.eswa.2018.03.050
- Berry, A. (1996). Improving production of aromatic compounds in *Escherichia coli* by metabolic engineering. *Trends in Biotechnology* 14:219-259.
- Bailey, J. E., Shurlati, A., Hatzimanikatis, V., Lee, K., Renner, W.A. and Tsai, P.E. (1996). Inverse metabolic engineering a strategy for directed genetic engineering of useful phenotypes. *Biotechnology & Bioengineering* 52:109-121.
- Follstad, B. D. and Stephanopoulos, G. (1998). Effect of reversible reactions on isotope label redistribution analysis of the pentose phosphate pathway. *European Journal of Biochemistry* 252: 360-371.
- Kacser, H. and Burns, J.A. (1973). The control of flux. *Symposium of the Society of Experimental Biology* 27:65104.
- Heinrich, R. and Rapoport, S.M. (1997). Metabolic regulation and mathematical models. In *Progress in Biophysics and Molecular Biology*, Vol. 32, Butler, J. A. V., Noble, D., Ed., Pergamon Press: Oxford, UK, pp 1-82.
- Savageau, M.A., Voit, E.O. and Irvine, D.H. (1987). Biochemical systems theory and metabolic control theory: I. Fundamental similarities and differences. *Mathematical Biosciences* 86:127-145.
- Brown, G.C., Hafner, R.P. and Brand, M.D. (1990). A 'top-down' approach to the determination of control coefficients in metabolic control theory. *European Journal of Biochemistry* 188:321-325.

Redman, M., King, A., Watson, C., & King, D. (2016). What is CRISPR/Cas9? *Archives of Disease in Childhood - Education & Practice Edition*, 101(4), 213–215. doi:10.1136/archdischild-2016-310459

Ghalayini M., Magnan M., Dion S., Zatout O., Bourguignon L., Tenaillon O., et al. (2019). Long-term evolution of the natural isolate of *Escherichia coli* 536 in the mouse gut colonized after maternal transmission reveals convergence in the constitutive expression of the lactose operon. *Mol. Ecol.* 28 4470–4485.

Kimple, M. E., Brill, A. L., & Pasker, R. L. (2013). Overview of Affinity Tags for Protein Purification. *Current Protocols in Protein Science*, 9.9.1–9.9.23.

Terpe, K. (2005). Protein Tags. In: *Encyclopedic Reference of Genomics and Proteomics in Molecular Medicine*. Springer, Berlin, Heidelberg . [https://doi.org/10.1007/3-540-29623-9\\_3650](https://doi.org/10.1007/3-540-29623-9_3650)