Protein Expression and lysis

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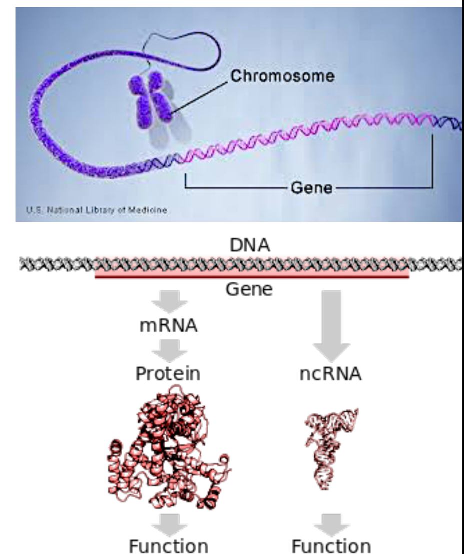
What is a protein expression?

- Recombinant **protein expression** refers to the manufacture of proteins derived from recombinant DNA.
- After proteins being expressed, they start to fold to make the 3D structure.
- Remember!
- **Genes** are made up of DNA.
- In biology, a **gene** is a sequence of nucleotides in DNA or RNA that encodes the synthesis of a gene product, either RNA or protein.
- Gene: A **gene** is the basic physical and functional unit of heredity.



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What is a gene expression?

- **Gene expression** is the process by which the information encoded in a **gene** is used to direct the assembly of a **protein** molecule.
- The cell reads the sequence of the **gene** in groups of three bases (as explained in the last lecture).
- In prokaryotic and prokaryotic, gene expression is regulated differently.
- **In prokaryotic**, Gene expression is regulated primarily at the transcriptional level.
- **In eukaryotes,** Gene expression is regulated at many levels (epigenetic, transcriptional, nuclear shuttling, post-transcriptional, translational, and post-translational).

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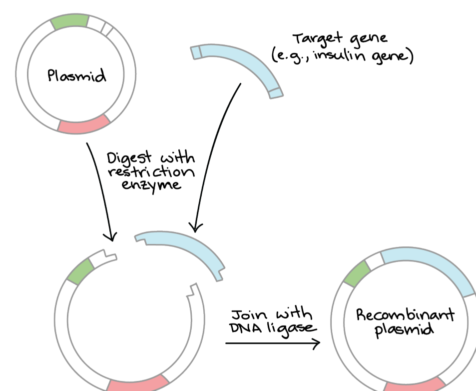
To express proteins, we need a Biotechnology

- What is a DNA cloning:
- It is making an identical copy for an organism.
- It refers to the process of isolating a DNA sequence of interest for the purpose of making multiple.
- In labs, vectors are is used a a host to make an identical copy for a specific gene.
- Then, this gene is hosted in *Ecoli* produce protein (outside of the living body).
- In vivo or in vitro?



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How does genetic engineering work?

- A plasmid ‘’ a sequence of DNA” is inserted to a vector which contain sites for polymerase binding that express a protein.
- Also, vector has an antibiotic sites to kill unwanted Bactria.
- It also has sites that can used to ligate or in-ligate (adhesive) the plasmid inside.
- It has also a site to induce the protein synthesis.
- Finally, vector is transformed to the Bactria ‘’a host ‘’ to synthesis the requested protein.

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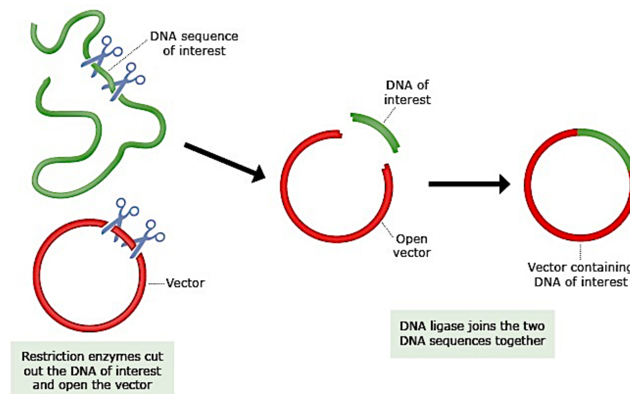
What is a genetic engineering?

- it is a removal of genes from one organism and insertion into another.



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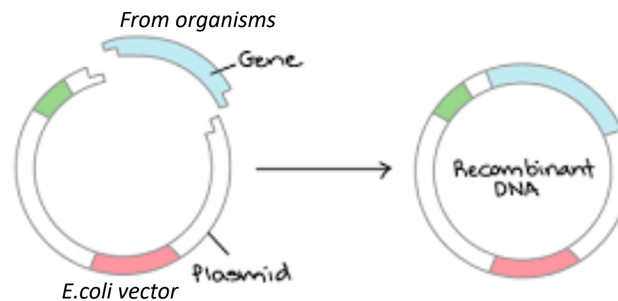
What is a recombinant DNA?

- It is DNA has been mixed with one of another species.



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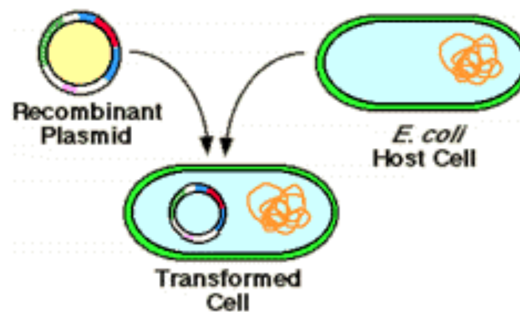
What is the transformation of a gene?

- It is a process used to transform a gene into a Bactria in order to express a protein from the selected gene.
- It is done by a adding the DNA to the Bactria and then heat shocking the Bactria at 42 ˚C in a water bath for up to 1 min only.
- Heat shocking process allow the Bactria wall to pass DNA inside it.



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The genetic engineering is done by PCR

- **What is PCR technique?**
- Using Polymerase chain reaction PCR in " three known steps" to generate ' to amplify ' thousands to millions of copies of a DNA from a very small amount of DNA.
- **What do you need for PCR?**

1. DNA template (the gene of interest).
2. Two primers 5' and 3'(oligo-nucleotides).
3. dNTP: Deoxy ribonucleotide triphosphate (each made up of deoxyribose sugar, phosphate group and N base).
4. Polymerase enzyme.

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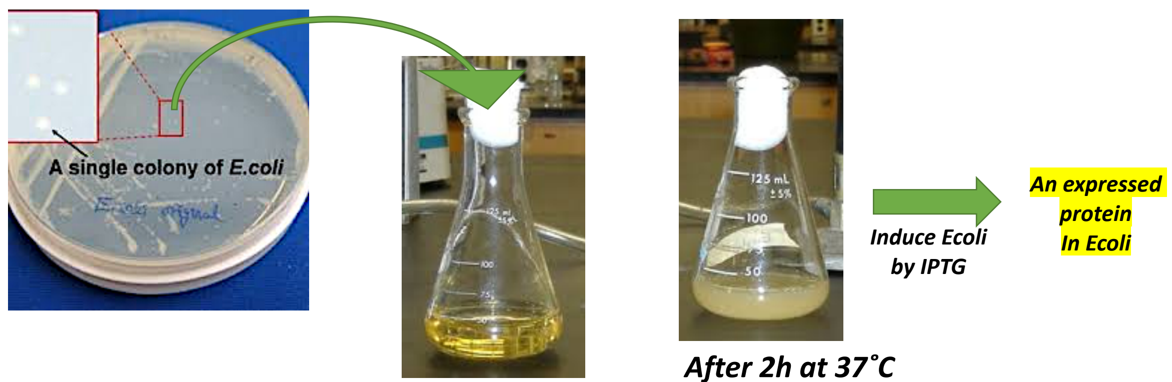
Making a growth, How?

- Once colonies of E.coli or any Bactria are grown on the plates, they can be placed into a growth media to generate millions of colonies and then induced them by IPTG to produce enough amounts of a protein.



After 2h at 37˚C

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What is the difference between an expressed protein and a tissue purified protein?

- **An expressed protein**: is a protein comes from a DNA recombinant gene transformed to a host such as Bactria or virus (Ecoli e.g). The host expresses the protein, then we can extract it and purify it in labs for experiments purposes.

- **A tissue purified protein:** is a protein comes from a specific natural tissue (muscle e.g) then it is only extracted and purified in the labs for experiments purposes.

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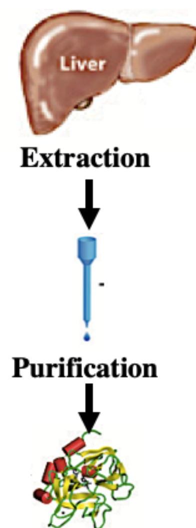
How to purify a protein?

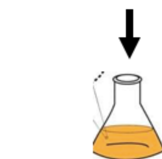
1. Tissue purified proteins.

- **We get a tissue directly from animals:**
- **E.g. Form muscle, cardiac ''heart'' Skin, Nails, hairs and other parts.**



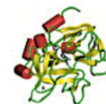
2. Expressed in E.coli.

Genetic engineering of DNA



Growth bacteria

Purification



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How do we extract the protein form E.coli?

1- Enzymatic lysis using lysozyme.

- We can use different types of biophysics and chemical methods:
- **Enzymatic lysis** is based on the digestion of the peptidoglycan layer of the bacterial cell wall by lysozyme.

- During cell lysis often a lot of DNA is released, it becomes necessary to add DNase (1 mg/ml) to reduce the viscosity of the preparation.

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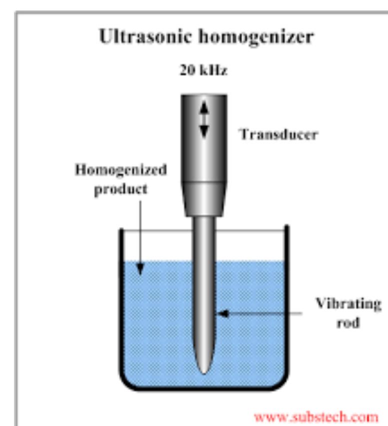
2-Sonication

Sonication : Ultrasound waves to disrupt the cell walls.

- Cells are lysed by liquid cavitation.
- What are the Problems of this method?
- Waves increase the temperature of solution which leads to protein degradation.
- So, the solution needs to be in ice always.



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3- Freezing and grinding.

An alternative lysis method is to freeze the cells directly in liquid nitrogen and ground the frozen cells to a powder.

• Liquid N2: -80 Celsius.

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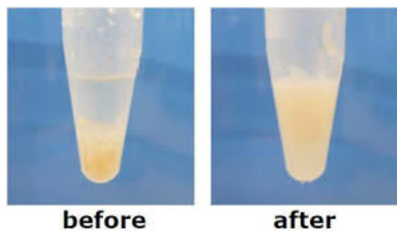
4- Homogenization:

- **Homogenization:** The presses lyse cells by pressurizing the cell suspension and suddenly releasing the pressure.
- **French press** homogenizer is used to compact the cell by 6000-10,000 psi (pressure) to disrupt the cell walls.



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What are the optimal conditions of the biomolecules? Protein?

- Physiological environments (as inside of a living cell) .

1. The temperature 37 °C.
2. The buffer range 7-7.2.

- So, a change on these factor will lead to:

1. Protein degradation. (mis folding)
2. Protein denaturation. (mis folding)
3. Protein aggregation. (mis folding)

- Other factor could affect the protein function and structure?.
- Increase:

1. The temperature 37 °C.
2. The buffer range 7-7.4.
3. Also, UV light radiation.
4. Digestion enzymes: e.g Trypsin.

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