PRINTERIA

Public engagement

Printeria: Biological handbook

Index

User interaction with Printeria

In Printeria we find **four types of basic DNA** parts that the user can combine in order to create transcriptional units. **But what is a transcriptional unit?**

A **transcriptional unit** is the combination of DNA sequences that allow the cell to transcribe DNA into RNA. If there is a **translating unit** forming part of the transcriptional unit, the result of the **transcription** is a **mRNA** to which a **ribosome** can be attached and the translation carried out: to form the **protein** encoded in the transcriptional unit.

Having explained this, **what parts do we need to form a transcriptional** unit that will lead our bacteria to produce the protein of interest?

- First we need a sequence that makes **RNA polymerase**, the enzyme in charge of transcription, bind to DNA. This sequence is the **promoter**.
  The promoter may be **constitutive**, allowing normal transcription without any special conditions, or it may be **inducible**.
  Inducible promoters require a protein called **transcription factor**, that by interacting with an external molecule can **activate or repress** the transcription of the transcriptional unit containing the inducible promoter.
  In addition, there are **promoters for other types of polymerases**, such as Heat shock protein (HSP) promoter that has affinity for a subunit of polymerase only present in shock conditions (σ^{32}), or as promoters that are recognized by **viral polymerases** (e.g., pT7, which is recognized by bacteriophage polymerase T7 and can sequester much of the cellular metabolism in pursuit of expression of the protein encoded by the transcriptional unit containing the promoter pT7).

  For all types of promoters, SynBio has developed variants with different affinities for RNA polymerase, allowing the number of transcripts produced by the cell to be modulated.

- Then we need the sequence that will recognize the ribosome in order to carry out the translation: the **ribosome binding site (RBS)**.
  As with the promoters, different RBS have been developed with different affinities for the ribosome, allowing the translation to be modulated.

- Then the sequence that indicates which protein will be formed is selected: the **CDS**. This sequence will indicate the synthesis of a molecule. For example, bacteria can express a reporter (so that the bacteria we are producing are fluorescent, coloured, etc.), or a transcription factor needed to complete a circuit.

- Finally, it is added the sequence that allows RNA polymerase to detach from DNA: the **transcriptional terminator**. It transcribed, but it is not part of the coding sequence to be translated into a protein sequence.

Once the **transcriptional unit** is designed **Printeria will start the reaction**.

Reactions in Printeria

Once the basic DNA parts to be assembled have been selected, they are deposited on the PCB where, the pre-digested destination plasmid, the two enzymes (the restriction enzyme and the ligase), the ligase buffer, and the BSA, that will prevent the enzymes from adhering to the surface, are added. All this reagents will form a **droplet of 15µl** that will be moved on the surface area of the **PC**B due to the **electrowetting effect**.

In the PCB, there is a zone at **37°C (hot zone)** and another at **16°C (cold zone)**. These zones are necessary to carry out the type of assembly we are going to do: a DNA assembly with Golden Gate Technology.

How does this type of assembly work?

In the Golden Gate Technology, it is used the **type IIS restriction enzymes**. They are a group of endonucleases that recognize **asymmetric double stranded DNA sequences** and **cleave outside** its recognition sites. Thus, digestion leaves short single stranded overhangs with **non-specific sequences**. As a consequence, using these enzymes you can cut a plasmid and paste the insert into another plasmid without regenerating the restriction site. This cut and paste is carried out by enzymes:

- The **BsaI Type IIS** endonuclease, which acts by cutting when the reaction is at 37ºC.
- The **T4 DNA ligase** which acts by bonding compatible cohesive ends when the reaction is at 16ºC.

During the reaction, **cycles of 37-16°C** are made so that in the area at **37°C** a significant part of the original plasmids are **cut**, leaving as a result the DNA basic fragments with the cohesive ends corresponding to each type of piece.

In the **16°C zone**, the **ligase** pastes the fragments in a way that two types of results remain:

- Regenerated vectors of origin, with recognition site for BsaI
- **Parts assembled correctly**, without site recognition for BsaI

In the next cycle at **37°C**, the already assembled parts are not cut, while the regenerated vectors are cut.
In this way, cycle after cycle, the number of original vectors is reduced, while **the parts are assembled in the destination vector.**

When the **assembly** is **complete**, the assembled genetic material is mixed with **electrocompetent bacteria**. The mixture moves to the exit area where there is an **electroporator**. The electroporator subjects the bacteria to a short electric shock of about **1600V** which **opens pores in the bacterial cell membranes** so that the genetic material can be introduced.
After electroporation, the bacteria are deposited in a culture tube with recovery medium. It takes **one hour** to **recover from** the **stress of the electric shock**.

After the bacteria have recovered, half **LB is added with the antibiotic**. The culture will be in this medium while its growth is monitored by measuring the OD_{600}. If the chosen protein is a fluorescent one, the protein expression can also be monitored.

Results and interpretation

From printeria we can obtain **fluorescence and OD_{600} data** with respect to time. The growth of the bacterial population will follow an exponential curve as indicated in the Monod model:

$$X(t) = X_o \cdot e^{\mu \cdot t}$$

Eq. 1. Monod model equation

Where:

- X is the amount of biomass, which varies with time. It is usually expressed in OD_{600} because the correlation of this data with the number of cells is linear.
- Xo is the biomass in time 0.
- μ is the specific cell growth and corresponds to $\frac{ln(2)}{td}$, being td the doubling time. Its units are, usually, min^{-1}.
- t is the time the culture has been growing, normally expressed in minutes.

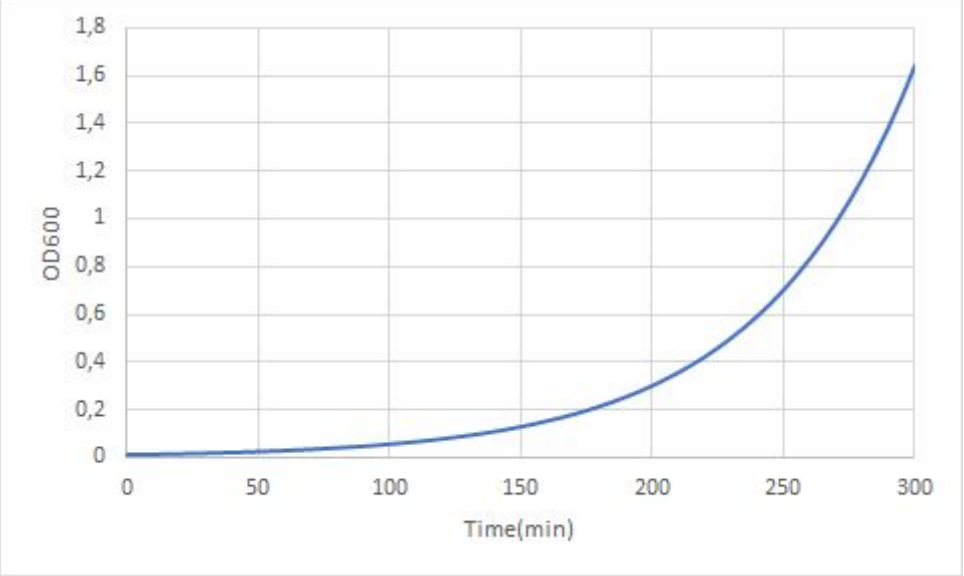

Figure 1. Expected cell growth following the Monod Model with μ=0.017 min^{-1} and Xo=0.01.

With regard to fluorescence, with constitutive promoters, the amount of proteins per cell is usually stabilised, giving rise to a linear correlation between fluorescence and OD_{600}. What the information gives us is the FOD (Fluorescence/OD_{600} ratio), which ultimately indicates the amount of proteins per cell.

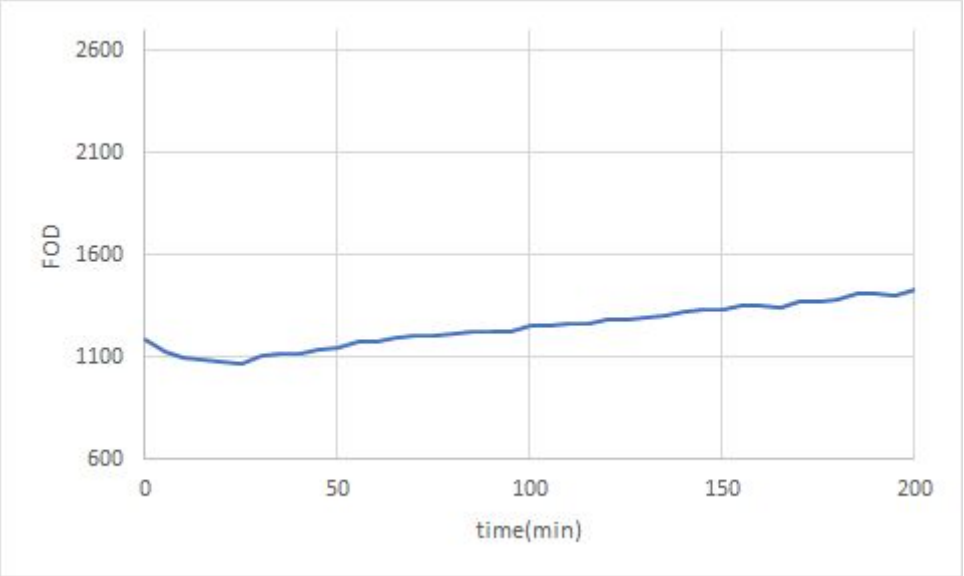

Figure 2. An example of FOD vs time graph obtained from experimental data.

For inducible promoters, the model that best fits the correlation between inducer concentration and expression for a greater number of transcription factors is the sigmoid or Hill function model.

$$F = \frac{1}{1+(\frac{K_A}{[L]})^n}$$

Being K_A the affinity constant of the inductor by the transcription factor (with units of inverse concentration), [L], the inductor concentration (with units of concentration) and n the coefficient of Hill.

For an inducible promoter regulated by an activating transcription factor, n is higher than 0 and the shape of the expected curve is as follows:

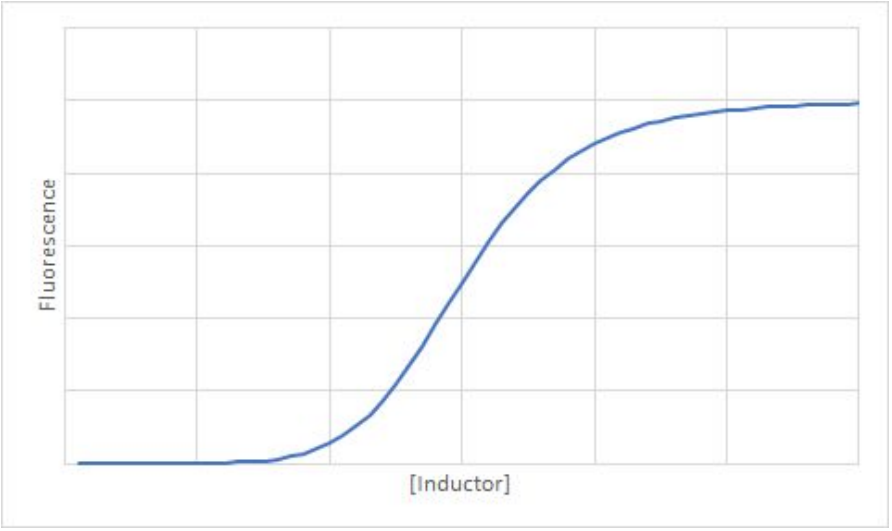

Figure 3. An example of a representation of the Hill equation with n>0. The effect of the inductor can be observed in the range in which fluorescence increases with the addition of more concentration of the inductor. With lower concentration, there is not enough inductor to activate

the transcription factor, while with higher concentration, the transcription factor is saturated and adding more inductor has no effect.

The curve for promoters regulated by a repressor corresponds to an n lower than 0:

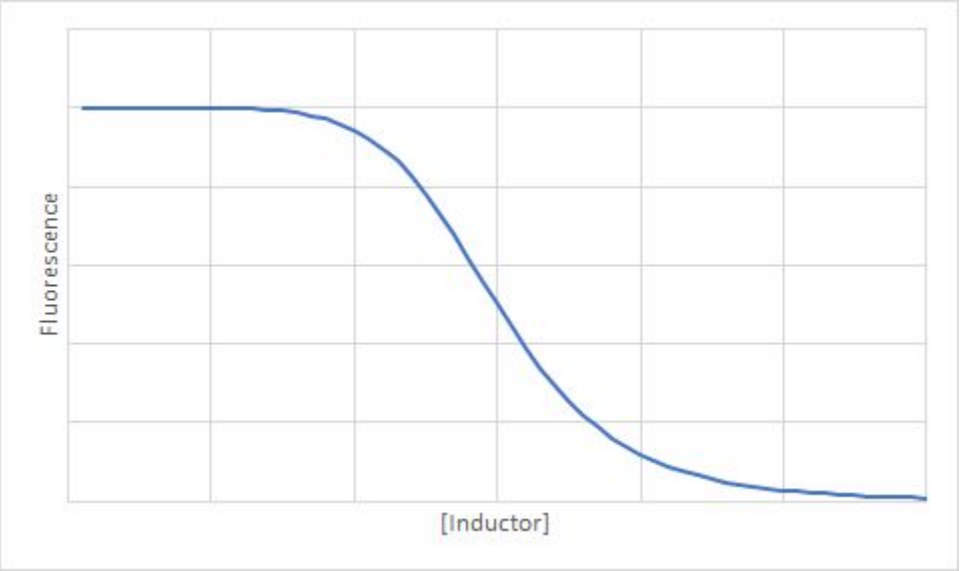

Figure 4. An example of a representation of the Hill equation with n<0.