BioRoboost!

Bacteria Everywhere

Teacher's Handbook

Contents

Introduction

Explaining synthetic biology to 15-16 year old high school students is a challenge, since several different complex items must be introduced: the concept of gene, the central dogma of molecular biology (transcription and translation), plasmids, and biological chassis.

Although some of these concepts are included in the high school curriculum, a comprehensive approach is needed to be able to convey to students not just a general idea of what synthetic biology is, but also of its relevance in today's society.

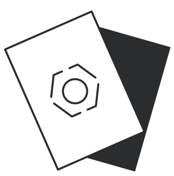
In this handbook we propose a didactic strategy with which students will be able to further develop these concepts in a gradual manner, and that will finally come together in a gamified activity (a card game) that will allow them to consolidate the concepts learned throughout the class.

Objective

The main objective of the present educational activity is for students to understand two concepts: **biological system** and **standardization**, both from a conceptual point of view and from the context of synthetic biology.

This didactic strategy is divided into two parts:

A previous theoretical explanation on standardization, and on the main components of biological pathways.



A game-based session in which students will put to practice the concepts learned during the first part of the session. By means of a card game, they will build a bacteria with a specific biological activity while using as many standard pieces as possible.

PART 1
Synthetic Biology and its parts

Section A: Bacteria everywhere

OBJECTIVE >> EXPLAIN THE CONCEPTS OF **BIOTECHNOLOGY** AND **SYNTHETIC BIOLOGY**.

THEORETICAL CONTEXT

During thousands of years, human beings have been taking advantage of the inherent capacities of certain living beings for their own benefit. For instance, to carry out processes like fermentation for the production of cheese or alcoholic beverages. We have also been able to select specific plant variants with interesting traits for the food industry, and cross-breed them. These examples can be considered the rudimentary beginnings of an increasingly popular discipline that, nowadays, is known as **biotechnology**.

This discipline focuses on the use of components or biological processes for obtaining products or solutions of interest for human beings. This includes the use of living beings in their original form (as they appear in nature), as well as organisms that have been genetically modified to provide them with abilities that they previously lacked.

Biotechnology faces this challenge by using a genetic "copy and paste". In other words, genes of interest present in certain organisms are identified, isolated and transferred to a receptor organism that will not only acquire the genetic information, but also the biological function that is encoded in the gene.

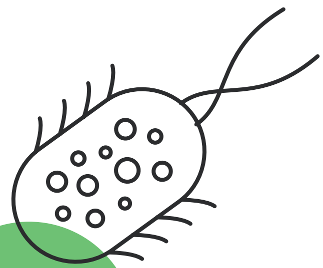
Nevertheless, during the past decade, a new branch of biotechnology known as **synthetic biology** has taken this process a step further. The goal of synthetic biology is to design and produce components and biological systems of human interest that didn't previously exist in nature. While traditional biotechnology is limited to the transfer of genes from one cell to another, synthetic biology focuses on how to build synthetic organisms or create new biological processes from scratch.

In this sense, synthetic biology can be considered the equivalent of **engineering** in the biological realm. It is a scientific discipline that has the potential to provide sustainable solutions for industrial processes that are currently unsustainable, to lower production costs of high-value compounds, or to tackle environmental issues such as oil spills. The goal of synthetic biology is to create biofactories that are able to undertake these tasks and many more.

In the same way as in engineering, synthetic biology considers the elements that compose a live organism as the pieces that compose a machine: these are combined to obtain a product that can be used for a specific task. The analogy can be applied at all organizational levels present in biological structures: from individual cells to tissues and whole organisms.

An example of simple biological systems that will be used in the present handbook are prokaryotic organisms and, more specifically, bacteria. These are unicellular organisms that are devoid of a cell nucleus and whose genetic modification is relatively simple.

Some applications of synthetic biology are based on including genes from other living beings –eukaryotes and prokaryotes– in bacteria by using circular DNA fragments known as plasmids, and also by manipulating the microorganisms, so they can transcribe and translate these exogenous genes as if they were their own, generating proteins or other compounds of industrial interest.



ACTIVITIES

First, the concept of biotechnology is explained as a discipline that uses biological systems and live organisms to generate products or processes of human interest. For this, four photographs are shown to the students: a yogurt, a plant, a beer, and pills.

Students are asked to raise their hand and vote in which products biotechnology has been used. It is likely that many students will fail, as biotechnology has been involved in the production of all of these products at some point or another. The role of biotechnology in each of these processes will be explained: fermentation (beer and yogurt), genetic selection (plants) and genetic engineering (pills).

Then, three more photographs are shown: a cosmetic cream, a petrol pump, and insulin injections. Students are asked which one of these products has been created by bacteria (insulin).

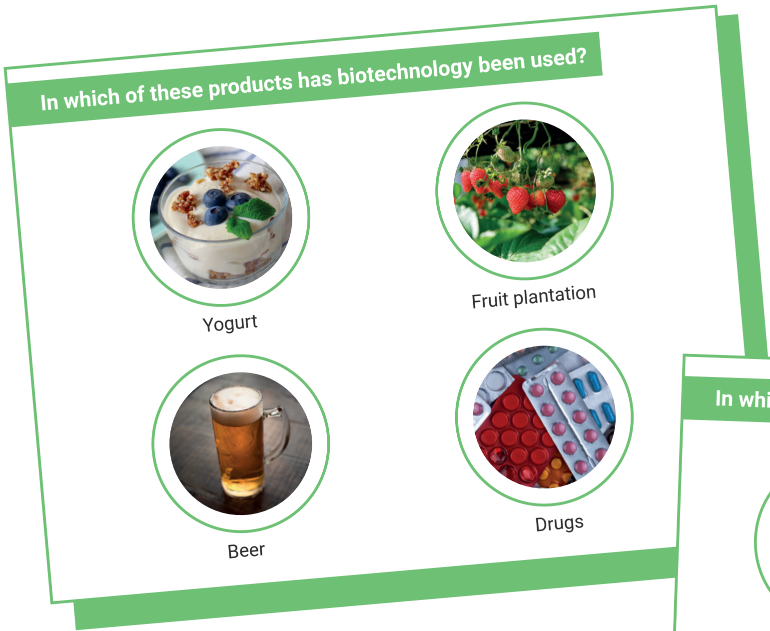
Reached this point, the concept of synthetic biology is explained as the engineering of biology, and its differences with biotechnology are highlighted. Synthetic biology allows to design and obtain live organisms with the ability to perform a process of interest or produce a compound of interest, in the same way that pieces of different materials are manipulated to obtain a car or another object.

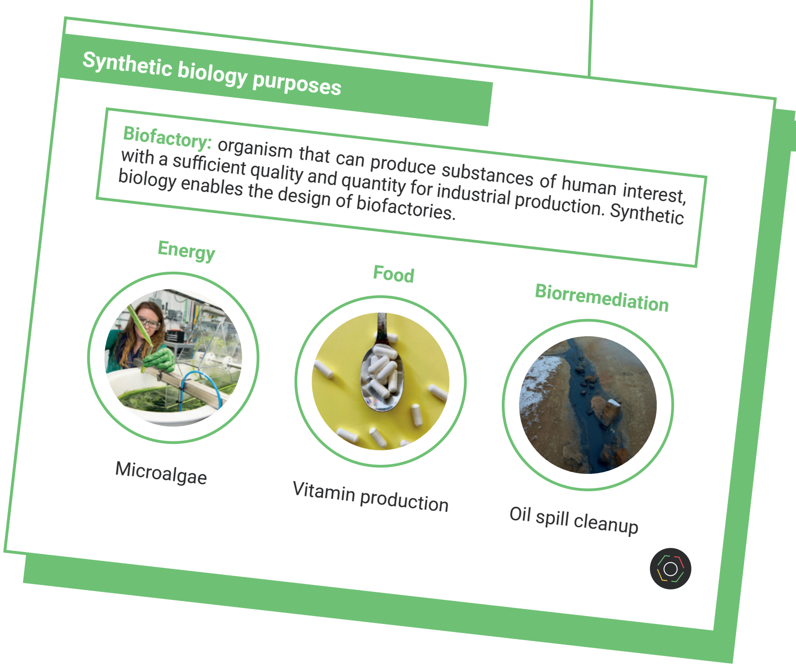
GRAPHICAL RESOURCES

A **quiz** that will accompany both questions for the vote by show of hands among the students. As an alternative, and only if possible within the available time and resources, there are interactive online software tools, such as mentimeter, that allow to add a dynamic component to this type of activities.

With these tools, the teacher will access a website in which the questions will appear, a code will be generated, and the students will introduce this code on the website to answer the question.

- Explanation of the concept of **biofactory** and several examples of synthetic biology applications: energy, food production, etc.





Section B: Mobile phones and bacteria

OBJECTIVE >> DESCRIBE THE BASIC PIECES THAT ARE NEEDED TO BUILD A SYNTHETIC ORGANISM.

THEORETICAL CONTEXT

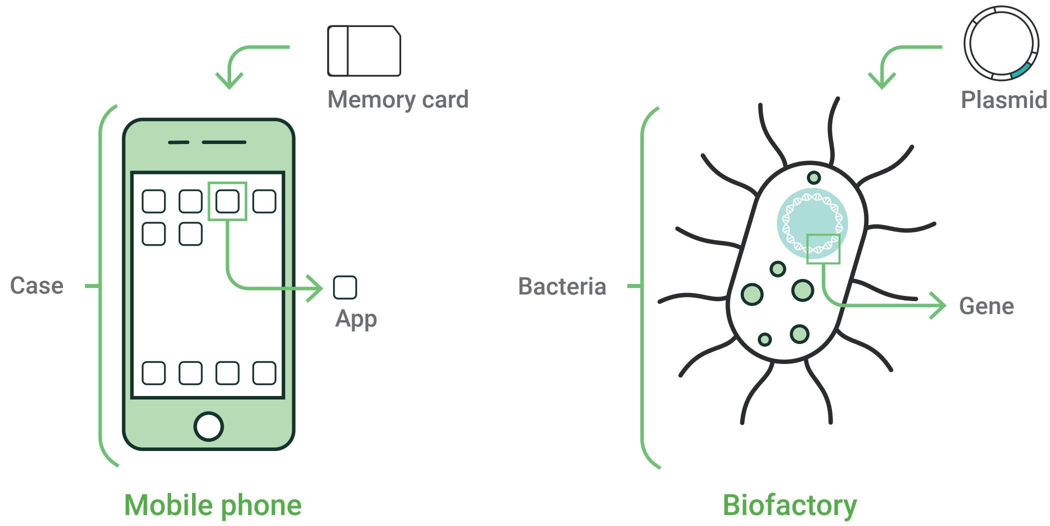
One of the similarities between synthetic biology and engineering is that both are based on the combination of individual pieces. These pieces are chosen based on desired function of the machine that is being designed. While in engineering these pieces are built with inert materials, in synthetic biology these pieces include the different genetic parts, the live beings that act as chassis and the vectors that are used to include the genetic information of interest in the live organism of choice.

This handbook works on the idea of synthetic biology pathways, with which an organism of interest can be obtained from the combination of pieces that are susceptible to standardization. With these pieces it is theoretically possible to design live organisms that are capable of undertaking almost any task or of producing a wide variety of molecules of human interest.

Although the pieces are different, both disciplines require a previous step to design the machine and to predict the way in which the pieces are going to interact. A basic synthetic biology construct is one in which exogenous genetic material is inserted into a bacterium with the intention of providing it with new capabilities of interest. The pieces that are needed for this genetic construct are: one or more genes that contain the genetic information required for the new functionality, a plasmid that will act as a vector to include this information in the bacterium, and a bacterium that will act as a chassis **(for further information on chassis, go to Glossary)**.

GRAPHICAL RESOURCES

- Comparison between a mobile phone and a biofactory using two drawings.
- Diagram of a bacterium in which the different parts of the synthetic constructs are shown. For each piece, the slide will show the corresponding card included in the card game.



ACTIVITIES

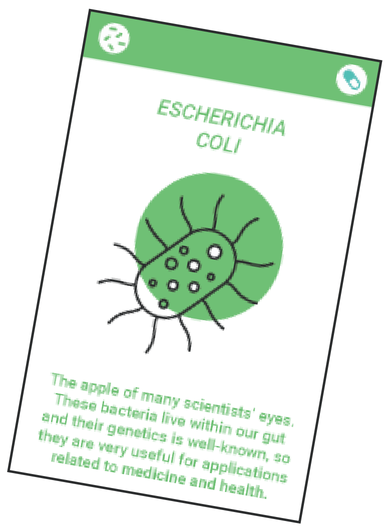
Comparison between a biological system and a mobile phone

In order to understand the different pieces that compose a biofactory, the design of a synthetic biology construct is compared to that of a mobile phone, in which every piece used has a function and, together, they generate a working system. For example, the mobile phone memory would be analogous to the bacterial chromosome, or the microSD card can be used to illustrate the concept of plasmid.

Removing the bacterial case

To explain each piece, the teacher can use the illustration provided in which a bacteria's interior is exposed. Each piece will be explained by using the cards from the BioRoboost game. This game includes a series of cards that represent the basic elements that compose a biological system based on bacteria. In order to use a less-abstract explanation, it is proposed to compare this to the process of cooking a dish.

CHASSIS



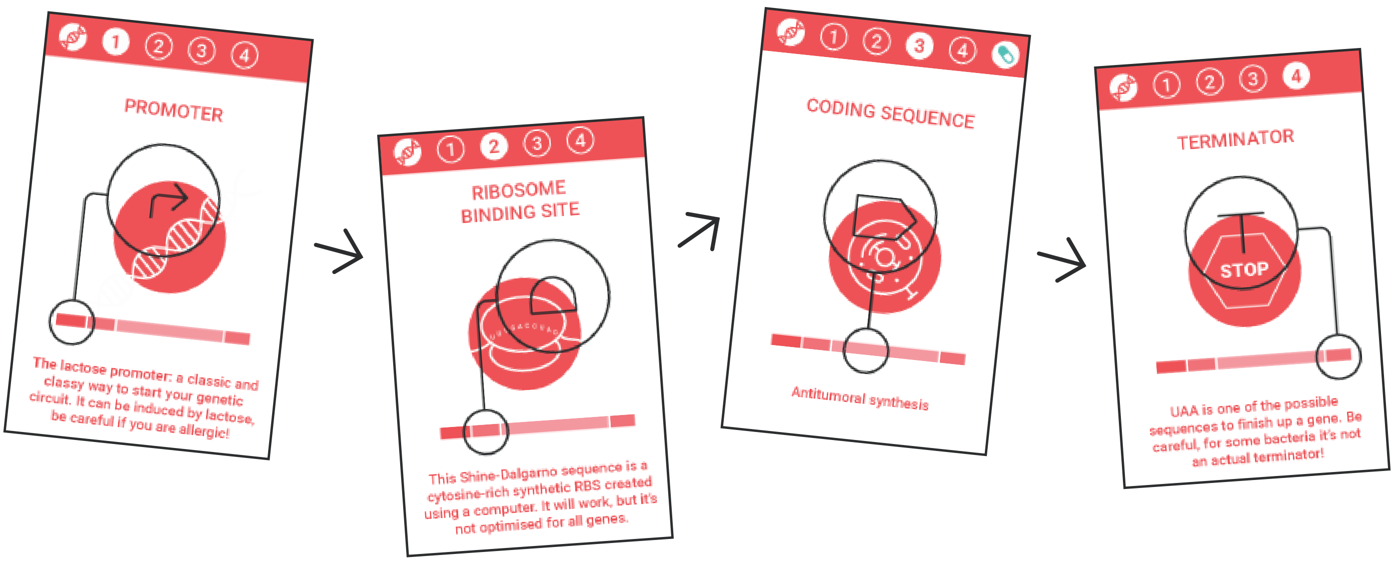
First, it must be explained that the first piece is precisely the organism that is going to develop the process, considering the "cook" who will produce our protein of interest. In synthetic biology, both prokaryotic (like *Escheriechia coli*, represented on the card) and eukaryotic cells (for example, *Saccharomyces cerevisiae*, also known as the baker's yeast) can be used, and even more complex live beings –such as multicellular organisms– can be modified.

GENE

The concept of DNA as the molecule that stores genetic information is explained, as well as the fact that the gene is the necessary information to generate a protein. Also, examples on the information that they may contain are given: genes that codify for the synthesis of a drug, for an enzyme that removes toxic compounds from the environment… Going back to the cooking analogy, the genetic information can be considered the recipe that the bacteria follows to produce the protein of interest.

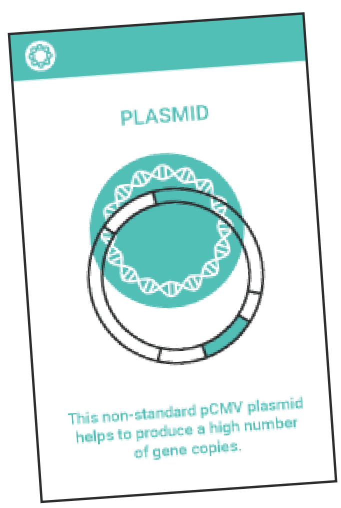
GENETIC CONSTRUCT

Genes have a series of components that enable them to be read by ribosomes. Continuing with the kitchen analogy, the genetic construct is the equivalent to the set of ingredients that the cook uses to create the dish. Furthermore, the central dogma of molecular biology is explained: the process with which DNA is transcribed to messenger RNA and, then, this is translated by ribosomes resulting in the creation of a protein. At this point, the overview that appears at the top of each card is explained, as it shows the position occupied by each element of the genetic construct: the promoter, where the start codon can be found; the ribosome binding site (RBS); the part that codifies for the specific activity of interest; and the terminator, where the stop codon can be found.



PLASMID

We need to use a "vehicle" to be able to introduce a gene into a live organism in the laboratory. An example is a plasmid. Continuing with the cooking analogy, the plasmid can be compared to the oven or the frying pan in which the ingredients are placed to perform the final dish.



PART 2

Standardization in Synthetic Biology

OBJECTIVE >> EXPLAIN THE CONCEPT OF STANDARD AND SHOW THE IMPORTANCE OF STANDARDIZATION IN SYNTHETIC BIOLOGY.

THEORETICAL CONTEXT

From the beginning of the Industrial Revolution, with the development of mass production, the use of **standards** has become a fundamental pillar to obtain **products with the same features and characteristics**, independently of who manufactures them and where they are manufactured.

Currently, standards are widely used in industry and in everyday life: for example, batteries are manufactured following a series of certified formats that are used by manufacturers worldwide. If each engineer used his or her own type of battery, it would be almost impossible to be able to buy the right one for your machine. At a more detailed level, standards refer to the adoption of a **common semantic and graphical language, the definition of measurement units for the features and parameters of the system**, the specification of the assembly of the systems components, and the implementation of non-ambiguous protocols to obtain these systems.

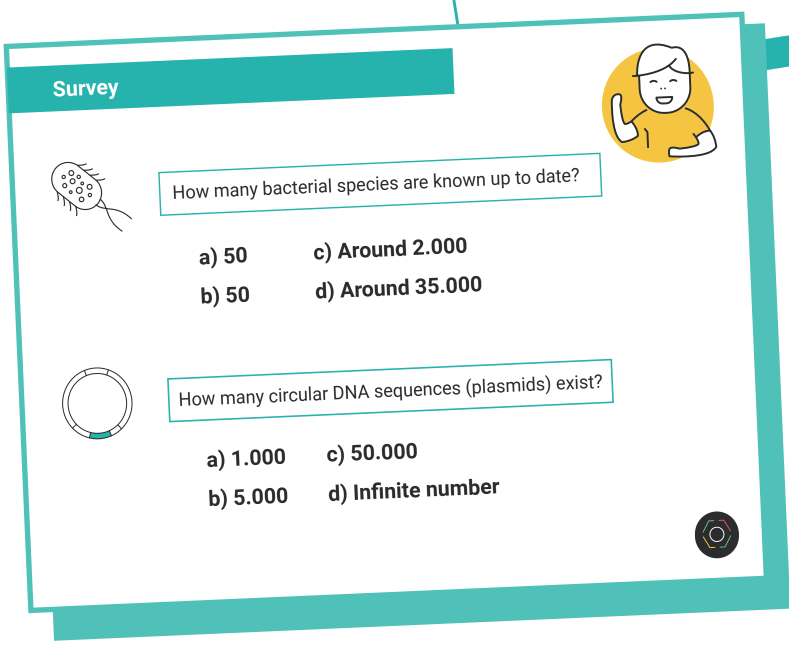
Although there are already many applications of synthetic biology, with this field experiencing a great deal of advances and successes in the last two decades, as of today there are still a series of obstacles that prevent exploiting all the potential of this discipline. One of the challenges of synthetic biology is precisely to characterize all the biological pieces and predict how they will behave when incorporated into a chassis.

There is a need to define certain standards and to promote their use for all the elements that are involved in a synthetic biology construct. In engineering, screws, electrical resistances, or current transformers are fully characterized and there are different certified models that the industry has agreed upon. In synthetic biology this has not yet been achieved, and it is still necessary for something similar to be set into place for bacterial strains, cell lunes, plasmid structure, or even the sequences that compose a genetic construct.

There is still a relatively large number of different plasmids, and of different bacterial species available for their use, and each laboratory chooses these pieces according to their own criteria. This hinders the reproducibility of experiments and limits the creation of new systems able to perform interesting functions.

GRAPHICAL RESOURCES

- A slide showing the options for each of the survey questions (see Activities on next page).
- A slide with an animation video.
- Drawings with two groups of scientists working in parallel and arrows representing the exchange of the bacteria.
- A slide with a torch and the definition of a standard.



ACTIVITIES

SURVEY

How many bacterial species are known?

a) 50 **b)** 200 **c)** Around 2.000 **d)** Around 35.000

Not all bacteria can be used in synthetic biology, seeing as we must be able to isolate them and grow them in a laboratory to be able to modify them. It is estimated that only 1% of the bacteria that inhabit the Earth are currently cultivable in laboratory conditions.

How many circular DNA sequences (plasmids) exist? Theoretically, there is an infinite number, as these are the result of the combination of the four nitrogenous bases that form DNA.

a) 1.000 **b)** 5.000 **c)** 50.000 **d)** Infinite

There is a large amount of pieces available to design a synthetic biology construct.

VIDEO

Two groups of scientists want to create a bacteria that is able to remove oil from the sea surface. Nevertheless, to be able to commercialize it, another group of scientists has to be able to build this same organism and ensure it can generate the same results. **What will happen when they try to build this bacteria?**

For a synthetic bacteria to be useful at a global scale, it must display the same features, and this can be done by using standard pieces. This is where the definition of standard is given, as a configuration that can be used as a model or reference for a certain object or piece.

THE TORCH GAME

To reinforce the concept of standard, the students are asked what elements a torch includes. The idea is that the students will point out the more simple elements that compose this object: light bulb, electric circuit, battery, plastic case. The teacher will have a torch with no battery, and several different batteries that won't fit in the torch, because these have to comply with a certain standard.

Standards are present in many objects, and students are encouraged to find examples:

SCREWS: there is a limited set of sizes and shapes that are widely used all over the world. **SOCKETS:** there are currently three main types of socket all over the world. If each architect decided to use a different type of current or her/his own socket design, the process of buying house appliances would be very complicated. **TIRES:** these have similar sizes, allowing them to be used in different types of cars. **USB PORTS:** these are standard connection points for the use of flash memories in almost every computer. What would happen if each computer used a different port? **MOBILE PHONE CHARGERS:** if each brand (or even each phone) used a different charger, replacing a broken charger would become a complicated endeavor.

PART 3
BioRoboost! Card game

GOAL >> TO BE THE FIRST PLAYER TO DESIGN A SYNTHETIC BIOLOGY CONSTRUCT USING THE BIOLOGICAL PATHWAY PIECES EXPLAINED DURING THE FIRST PART.

GRAPHICAL RESOURCES

- Cards used in the game. The genetic construct parts (red cards) are analyzed, and the line of numbers at the top (representing the order in which they appear) is discussed.
- Analysis of the icons that appear on the coding region cards and on the bacteria cards, which indicate for what type of application they can be used.
- Game rules. This slide will be maintained throughout the second part of the activity, so that the students can use it as a guide.

ACTIVITIES

Different cards and their key colours are shown: **red** for the pieces of the genetic pathway, **blue** for the plasmids, **green** for bacteria, and **yellow** for action cards. The icons at the top of each card are also highlighted, which allow to identify the type of card in case of doubt.

Also, the cards representing the pieces of the genetic pathway include a series of numbers indicating their physical position within the construct, to help students know if they have all the pieces of the circuit.

The goal of the game is not only to build a synthetic biology system, but to do so using the largest possible amount of standards. For this, the standard versions of the plasmid, promotor, RBS and terminator cards are shown. It is highlighter that the coding part cannot be standardized as there is only one sequence that codifies for each product.

It is also shown that the three bacterial cards correspond to those most frequently used in synthetic biology, but that each of them can be considered standard for a certain application. In this sense, *Escherichia coli* is usually used for medical-related applications; *Bacillus subtilis*, for industrial applications; and *Pseudomonas putida*, for environmental applications. Therefore, the bacteria of choice for our design must depend on the type of activity we want it to have. The icons on the left side of the card indicate the kind of application our coding regions and bacteria are for.

GAME RULES

- The goal is to use synthetic biology to design a bacteria that is able to perform a specific activity, and that contains more standard pieces than the bacteria designed by the rest of players
- Once shuffled, each player receives 6 cards. The rest of the cards are placed facing down in the center. In their turn, the players must take a card from the central deck. Before finishing their turn, each player must: (1) hand over a card of their choice and facing up to the next player; and (2) discard a card, also facing up, to the discard deck. At the end of every turn, a player can have a maximum of 6 cards in their hand.
- At the beginning of his/her turn, before taking a card from the central deck or playing an action card, a player can consider that he/she has built the best bacteria, and can put an end to the game. At that moment, all players must show their cards, and whoever has not collected all the pieces of the biological construct will be disqualified.
- A construction is considered complete when it contains all the pieces and when the coding region is compatible with the type of bacteria.
- The players who succeeded in completing their construct determine which one contains more standard pieces, and the winner will be the player with the highest number of standard pieces. Several players may tie.

Glossary

PLASMID: a circular DNA fragment that replicates autonomously, independently of the chromosomic DNA. Plasmids are present mainly in prokaryotic organisms (bacteria and archaea) and, under natural conditions, they don't usually contain essential genetic information, but rather they include genes that are, on many occasions, related to stress resistance (for example, antibiotic resistance). Their role in laboratories is to function as vehicles for the introduction of exogenous DNA sequences into carrier cells, in order to give them functionalities that they would not normally have.

GENE: DNA sequence that contains the necessary information to produce RNA and proteins. Genes also represent the molecular unit for genetic inheritance, as genes store the genetic information that is transmitted to the offspring.

From a simplistic point of view, a gene requires the following elements to be able to carry out its biological function:

- **PROMOTOR:** part of the genetic sequence that controls the initiation of the transcription process, either by promoting the expression of the gene or by suppressing it. This sequence is located before the DNA base triplet (ATG) that initiates translation. The promoter is important because it regulates the binding of the protein that uses DNA to synthesize messenger RNA.

- **RIBOSOME BINDING SITE (RBS):** part of the sequence that is located just before the start codon and that allows for the ribosome to bind to the messenger RNA and begin translation. This structure usually contains the 5'-AGGAGG-3' sequence known as the Shine-Dalgarno sequence. In general, this part doesn't translate and, therefore, is not a part of the protein that is synthesized after this process.

- **CODING SEQUENCE:** part of the gene that contains the information on which amino acids are going to constitute the protein that is encoded in the gene.

- **TERMINATOR:** part of the genetic sequence that indicates the end of the gene and that contains the translation stop codon (UAG, UAA, UGA). This sequence mediates the detachment of the ribosome from the messenger RNA, stopping the translation process.

VECTOR: DNA molecule that is used to transfer exogenous genetic material to a receiver cell, in which this genetic material can be replicated and/or expressed. Although there are several types, plasmids are the most frequently used vectors.

CHASSIS: in synthetic biology, this concept refers to the cells in which new genes can be introduced to create products of interest. They are live cells that possess the basic features to survive (for example, ability to produce energy), and that can be used as a starting point to introduce exogenous genes with the aim of introducing new functionalities.

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement N820699.