

# MICRO BIO WORLD

A STRUGGLE FOR LIFE

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# 1- Basic Rules

## Context

MicroBioWorld is a card game dedicated to microbiology. This game is based on current scientific knowledge and offers you a trip into the world of microorganisms in order to significantly improve your grasp of the existence, diversity and manipulation of these microorganisms.

MicroBioWorld was created by students as part of the iGEM competition (= international Genetically Engineered Machine) and is accessible for all players, from the age of 1, to play with their family or friends.

## Goal

Every player acts as a bacteria colony (a cluster of bacteria originating from the same mother bacterium).

To win, be the first to build a colony of 10 log, being ten billions of bacteria! To do so, you can use the benefits granted by your type of bacteria and the modifications you add to it. There are also other paths to victory, but you will discover it by yourself along your journey.

## Playing a game turn

### **Preparation :**

Each player receives one bacteria card. Every player as to put his bacterium card visible as well as all its equipped plasmids. “Media” cards are mimicking the environment in which colonies are growing during the game (see paragraphs about “media” for further information). The game starts with the

LB (Lysogeny Broth) media. Shuffle all the other media cards and place the pile face down on the table.

Then, shuffle the stack.

From this stack, hand out a deck of 3 cards to each player (the following effect isn’t active: *“this card has to be played immediately”*).

Every player starts with 5 log bacteria, which accounts for one hundred thousands bacteria. Indicate your progression with a little pointer provided in the game.

### **Turn :**

When it is your turn you can play any card of your hand.

Then, do one action among the three following:

- **Draw a card.** Unless otherwise indicated, a drawn card must stay in the player’s deck for one turn before being played, and the deck can contain up to 3 cards. You can discard a card to draw another one from the pile.

- **Multiply** (the colony gains 1 log)

*Learn more:* A bacterium can develop by using resources of the medium (carbon, nitrogen, iron, etc.). Within a colony, bacteria are multiplying by creating a copy of themselves: they are dividing. In the game, the “log” are used to describe the amount of bacteria. This notion is used to describe the order of magnitude of a bacterial population that can reach billions of individuals. For example, in the large intestine, home to many bacteria, there are approximately 14 log bacteria, meaning a hundred of thousand of billions of bacteria !

- **Attack** an opponent colony (- 1 log to the attacked colony)

*Learn more:* Some bacteria have a whole arsenal to limit the growth of their competitors (for example by trapping for some resources like iron) or to destroy them! (for example by producing toxins) However, although in

the game all bacteria are able to attack any other one, it is not the case in nature since they are rarely in competition.

## 2 - Bacteria cards (6 cards)

**Rule:** at the start of the game, each player receives a random bacterium card that defines the type of bacteria that he is going to embody. The player has to take advantage of each bacterium's strengths and weaknesses to be the first reaching 10 log.

**Learn more:** bacteria are microscopic living organisms (between 0.2 et 2  $\mu\text{m}$ ) and are only composed of one single cell. Currently, we estimate more than billions bacterial "species" occupying almost all environments (from freezing areas to hot deserts, from human guts to marine abysses...).

Bacteria have wide ranges of properties, shapes and behaviours.

Some are rod-shaped (*bacillus*), others round-shaped (*coccus*).

Prototrophic bacteria are able to feed themselves in any environment, while auxotrophic ones need to get all nutritive elements from their environment to grow. Some bacteria are able to cause diseases to humans, we call them pathogens. The scientific community has also classified bacteria according to their ability to be stained by the Gram technique that depends on the composition of their cell wall (they are called either Gram-positive or Gram-negative).

On agar medium, bacteria can multiply themselves and end up forming a visible cluster (around 8 log). This small dot is called a colony and is going to grow bigger and bigger as long as the nutritive elements are sufficient to sustain its growth. The color and the shape of this colony depends on bacteria.

### *Bacillus subtilis* (1 card)

#### **Distinctive feature: Sporulation**

Once in the game, when your colony is undergoing damage, *B. subtilis* can sporulate: you do not lose any log during this turn.

**Characteristics:** Bacillus, Gram-positive, non-pathogenic, prototrophic.



**Learn more:** Sporulation enables some organisms to survive a wide range of stresses.

To do so, the bacterium enters a vegetative state called dormancy and surrounds itself with a cell wall, protecting it from outside aggressions. When the outside conditions improve, the spore can "sprout" and the bacterium can resume its growth. Scientists were able to make spores from egyptian mummies sprout!

### *Deinococcus radiodurans* (1 card)

#### **Distinctive feature: Polyextremophile**

*D. radiodurans* can divide itself at every temperature and is resistant to UV radiations.

**Characteristics:** Coccus, Gram-positive, non-pathogenic, auxotrophic.



**Learn more:** *Deinococcus radiodurans* is a polyextremophile bacteria that is able to

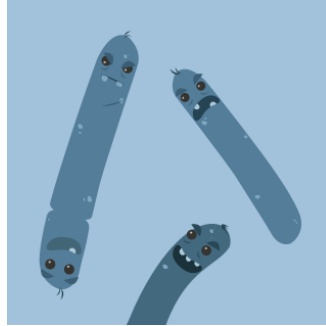
maintain its division on a wide range of temperatures. Thus it is of great interest for the scientific community to understand its survival capacity. This capacity is thought to be due to the multiple copies of its genes.

## *Escherichia coli* K12 (1 card)

### **Distinctive feature: Microbiota**

Once in the game you can chose another player to be part of *E. coli* microbiota: you can no longer attack or be attacked by this player. Be the first to reach 10 log to defeat your microbiota.

**Characteristics:** Bacillus, Gram-negative, non-pathogenic, prototrophic.



**Learn more:** *Escherichia coli* is naturally very abundant in human guts, it is even the first organism to appear in it and colonize it at birth. It is a facultative aerobic, meaning that it can develop with or without oxygen. Because of its easy-to-grow capacity it has been the most widely used bacterial model in laboratories and bio-industries to produce different organic compounds.

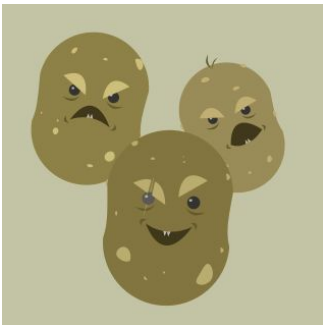
## *Staphylococcus aureus* MRSA (1 card)

### **Distinctive feature: Multiresistance**

*Staphylococcus aureus* MRSA is chloramphenicol and ampicillin resistant.

**Characteristics:** Coccus, Gram-positive, pathogen, auxotrophic

**Learn more:** Its common name is golden staph due to the pigments it is producing



that give a gold color to its colonies. For humans, it is naturally present on epidermis. This bacterium is well-known for its pathogenic behaviour when an infection opportunity is declared, in case of a wound for example. MRSA (Multi Resistant Staphylococcus Aureus) variant is sadly famous for its capacity to resist to most antibiotics.

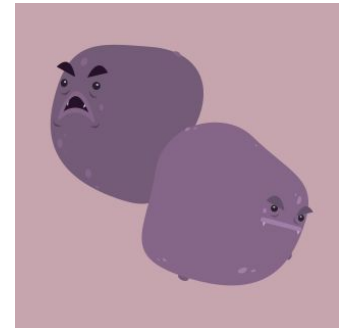
## *Streptococcus pneumoniae* (1 card)

### **Distinctive feature: Competence**

Once in the game, when you attack another colony or when you're attacked by another colony, you can steal a plasmid card from it. Put this plasmid on your hand, you can have up to 4 cards in your hand.

**Characteristics:** Coccus, Gram-negative, pathogen, prototrophic.

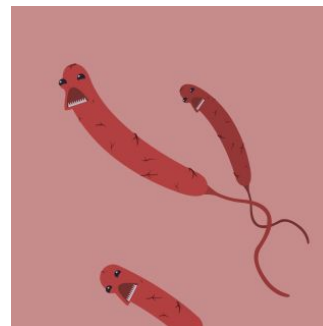
**Learn more:** *S. pneumoniae* is a human pathogen found in multiple infection, (pneumonia, ear infection, meningitis...) The competence mechanism of *Streptococcus pneumoniae* allows the bacteria to naturally take on plasmids of other microorganisms (see the paragraph "plasmids" for further information).



## *Vibrio cholerae* (1 card)

### **Distinctive feature: Cholera toxin**

During your attack, cholera toxin is spread in the opposing colony: if the attacked colony has at least 7 log, *V. cholerae* can also divide in addition to the attack.



**Characteristics:** Bacillus, Gram-negative, pathogen, auxotrophic.

**Learn more:** Vibrio cholerae is a bacterium that lives in water and causes the cholera disease. If it is consummated by human, it disrupts intestinal system and causes diarrhoea, then joins other water courses, allows it to colonize other environments. It also has a cutting apparatus (the type 6 secretion system), enabling it to attack other microorganisms to take advantage of their resources.

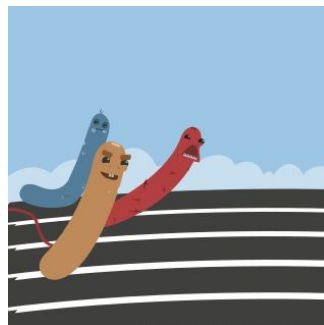
### 3 - Media cards (9 cards)

“Media” cards mimick the environment in which colonies are growing during the game. The “Transplanting” card allows players to change the culture media during the game: when it is played, take the next card of the media stake, it becomes the new common media for all players.

**Learn more:** in laboratories microorganisms are grown on growth media, containing all the necessary nutrients for bacterial growth. The agar media is packaged in a Petri dish, a small round plastic box. If an antibiotic is added to a medium, this medium is called an selective medium: only the bacteria with the corresponding antibiotic resistance can grow on it. Some media are said differential, they color the bacteria according to their characteristics.

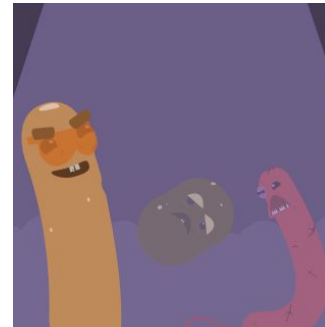
#### 25°C LB medium (or Lysogeny Broth) (1 cards)

**Distinctive feature:** bacteria divide and attack in the normal way. In the game it is the starting growth medium.



**Learn more:** It is composed of yeast extract, peptone (mix of small proteic molecules that provides a nitrogen source among other things), salt and water.

#### 25°C LB medium under UV radiations (1 card)



**Distinctive feature:** at the beginning of your turn, you lose 1 log before any other action.

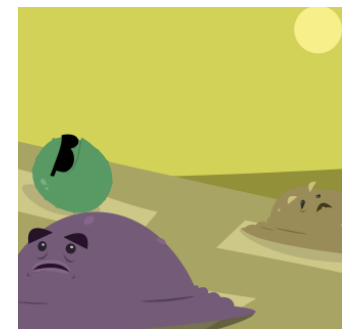
**Learn more:** Few mutations naturally occur in every genome. Ultra Violet (UV) radiations are highly mutagenic agents: they stimulate the apparition of a lot of mutations, leading to an alteration of the genetic information. If too many mutations occur, the bacteria can't keep

their vital functions.

#### 45°C LB medium (1 card)

**Distinctive feature:** all non-thermophilic bacteria cannot divide. This card promotes thermophilic organisms.

**Learn more:** Thermophilic microorganisms are adapted to high temperature areas like subsea hydrothermal ventings. High temperatures increase the fluidity of membranes and inactivate some of molecules. Therefore, these microorganisms use different fatty acids to maintain the integrity of their membrane. Thermophilic organisms also



contain thermoresistant proteins with a more compact and stable structure.

### 25°C LB medium + Ampicillin (1 card)

**Distinctive feature:** all the non-resistant colonies lose 3 log.

*Learn more:* Ampicillin is a bacteriocin antibiotic, killing the bacteria of the penicillin family. This antibiotic blocks the peptidoglycan production, one of the elements of the cell wall. To do so, it attaches to the enzymes that form the bonds between the molecules forming the peptidoglycan.



Players can attack the fungus during their turns. If it dies, the media reverts to LB. If it reaches 10 log, it wins the game ! The media cannot be changed, and transplanting allows players to restart the fungus to the number of players + 1 log.



*Learn more:* Microorganisms can live nearly everywhere. The microbiologist's work is done under sterile conditions to avoid any unwanted contaminations. Even if done in the proper conditions, contaminants can grow, sometimes even in a selective medium. This contaminant can rapidly invade the culture media.

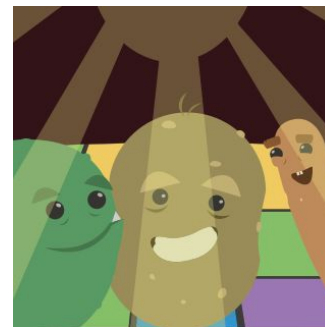
### 25°C LB medium + Chloramphenicol (1 card)



**Distinctive feature:** the non-chloramphenicol resistant bacteria cannot divide.

*Learn more:* Chloramphenicol is a bacteriostatic antibiotic: it blocks the division of bacteria. It inhibits the production of the proteins needed for division.

### 25°C Chapman medium (1 card)



**Distinctive feature:** only the Gram-positive bacteria can divide.

*Learn more:* Chapman medium is enriched in salts that promote the growth of Gram-positive bacteria, thus separating them. Moreover it can reveal the presence of colonies thanks to a colored marker. This change of color is achieved thanks to the fermentation of mannitol (a natural sweetener used as a nutrient substrate)

which acidifies the medium, this acidity is revealed thanks to a dye sensitive to acidity (phenol red).

### 25°C Contaminated LB medium (1 card)

**Distinctive feature:**

A fungus has contaminated the growth medium, its initial log is equal to the number of players + 1. It divides at each round.



## 25°C Milieu McConkey (1 card)

**Distinctive feature:** only the Gram-negative bacteria can divide.

*Learn more:* McConkey medium is used to separate Gram-negative bacteria. It contains crystal violet and bile salts that slow down the growth of Gram-positive bacteria. Then like mannitol in McConkey medium, the fermentation of lactose acidifies the medium, allowing to detect more easily the colonies thanks to a dye sensitive to acidity.



## 25°C Minimal medium (1 card)

**Distinctive feature:** auxotrophic bacteria (those that cannot synthesize all their molecules) cannot divide.

Auxotrophic bacteria need their environment to contain all the nutrients for their growth because they cannot synthesize all molecules that allows them to divide. For example, amino acid or a vital lipid need to be added to the culture

media..

## 4 - Plasmids cards (24 cards)

**Rules:** Plasmids cards are assets you can add to your bacteria during your turn. It remain attached to your colony during

the game (expect special effect). They are 3 plasmid family (red, blue, green). You cannot equip your bacteria with two plasmids of the same family. If you want to change a plasmid for another one of the same family, you need to pick randomly one of the two cards to equip your bacteria and discard the other one.

*Learn more:* Plasmids are round little DNA molecules. Like chromosomal DNA, they own genes, that give new abilities to bacteria that get the plasmid. Plasmids are able to replicate themselves into bacteria, that means generate the synthesis of another similar plasmid thanks to the proteins inside the bacterium. Plasmid replication is possible thanks to a special DNA sequence called the ORI sequene (for replication origin). They are several ORI families and plasmids with the same ORI can't coexist in one bacterium, one of them will be randomly lost. This phenomenon is called plasmid incompatibility.

## pTox: "Yeev toxin" plasmid (2 cards)

**Distinctive feature:** The colony you attack cannot divide during the next turn.

*Learn more:* The cytoskeleton is a structure made of filaments that maintain the cell size, internal organization and mechanical properties. It is a crucial element, needed for cell division. The YeeV toxin inhibits the enzyme that assembles the filaments of the cytoskeleton, thus blocking cell division. In eukaryotes, like humans or mammals, it is the phalloidin that blocks the cytoskeleton formation. This toxin was taken from Amanita phalloides, a fungus.



### pBIO: “Biofilm” plasmid (1 card)



**Distinctive feature:** You only lose 1 log when damaged.

**Learn more:** Microorganisms are able to develop forming a compact and structured pile called biofilm. This type of growth allows the bacteria it contains to respond to external stress (physical attack, acidity, toxins, antibiotics...) like a uniform tissue, by establishing complex communication systems within the biofilm itself. This is a

very efficient defence strategy to protect microorganisms against an hostile environment.

### pRK2073: “Conjugation” plasmid (2 cards)

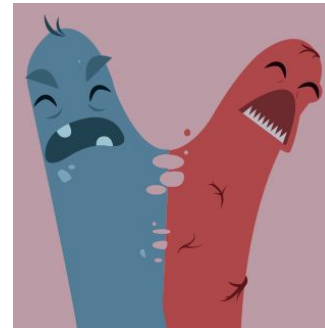
**Distinctive feature:** During your attack phase, you can steal a plasmid card from your opponent’s colony.

Put this card in your hand. You can have up to 4 cards in hand.

**Learn more:** Conjugation is a mechanism that allows a bacterium to transfer plasmids to another one. A donor bacterium possesses a plasmid, and a receiver one is able to initiate conjugation, and will receive the plasmid from the donor. In the game, the “conjugation plasmid” means your colony is the receiver.



### pSymb: “Symbiosis” plasmid (1 card)



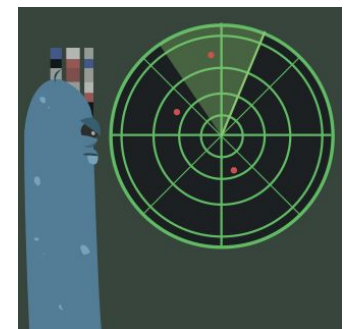
**Distinctive feature:** Choose a colony to be symbiotic with. Each time one of the symbiote divides, the other also divides. This effect does not apply to special division cards (fructose, xylose, glucose or any special division of a bacteria card). If the culture media inhibits the growth of your symbiote, it move of 1 log if you divide yourself.

**Learn more:** Over time, living organisms have developed different strategies to fit best specific living conditions. For this purpose, some of these organisms evolved to live together so that they benefit from each other, this is called symbiosis.

### pQS: “Quorum Sensing” plasmid (1 card)

**Distinctive feature:** you cannot be attacked twice in a row.

**Learn more:** For a bacterial colony, it is crucial to detect and be detected by other bacteria in the same environment. To do so, they produce specific molecules that are sensed by other bacteria. Then, depending on the molecules that are sensed, a colony “knows” which bacteria are around. This phenomenon, called quorum sensing, allows bacteria to





activate different mechanisms in order to unsettle competitive bacteria, or to better protect themselves.

### pSST6: “Type VI secretion system” plasmid (1 card)



**Distinctive feature:** Your attacks cause twice as much damage.

**Learn more:** Bacteria have systems to excrete molecules from inside to outside, or even directly inside the opponent bacteria. Type VI secretion system (SST6) is an example of that. It is a structure that looks like an hollow needle able to inject toxic molecules inside a target cell.

### pBR322: “Ampicillin resistance” plasmid (2 cards)

**Distinctive feature:** Your colony is no longer impacted by Ampicillin.

**Learn more:** This plasmid contains the ampicillin resistance gene (*bla* gene) that produces the  $\beta$ -lactamase enzyme, able to cut ampicillin molecules, thus inactivating its antibiotic effect.



### pSB1C3: “Chloramphenicol resistance” plasmid (2 cards)



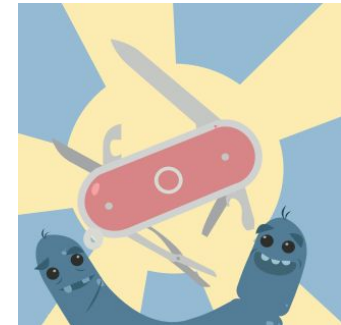
**Distinctive feature:** Your colony is no longer impacted by chloramphenicol.

**Learn more:** This plasmid contains the chloramphenicol resistance gene (*cat* gene) that produces the acetyltransferase enzyme, able to add chemical groups to chloramphenicol molecules, thus inactivating its antibiotic effect.

### pAdapt: “Adaptation” plasmid (2 cartes)

**Distinctive feature:** you can draw a card and attack a colony during your turn.

**Learn more:** when a colony are close to other bacteria, it must elaborate new strategy to avoid attacks or pressure of competitor. In this context, those who can adapt themselves quickly are advantaged. Adaptation is defined by the gain of a new function / of a new gene (in the card game, it's illustrated by picking a card).



## pAMP: “Antimicrobial peptides” plasmid (2 cartes)



**Distinctive feature:** During your attacks, you can excrete antimicrobial peptides aiming to a second target colony: it loses 1 log.

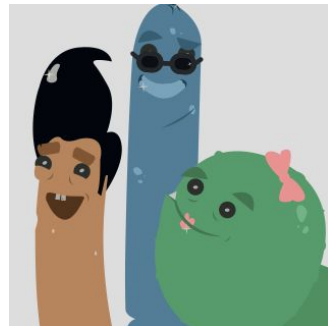
*Learn more:* this plasmid contains the genes for antimicrobial peptides, capable to create pores into the bacterial membrane. The cytoplasm (inside the bacteria) drains outside the bacteria, and the bacteria dies. Those peptides are

usually produced by other organisms to fight against infection of pathogenic bacteria.

## pChiadé : “Chiadé” plasmid (4 cards)

### Distinctive feature:

- With 1 chiadé plasmid, nothing happens
- With 2 chiadé plasmids, you can do 2 actions at your turn (you can do twice the same action)
- With 3 chiadé plasmids, you can do 3 actions at your turn.

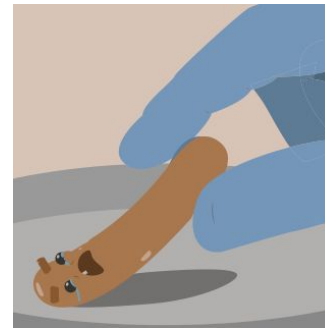


*Learn more:* In synthetic biology, this French word qualifies a complex and highly regulated genetic system that confers badass effects. Synthetic biology allows to create new functions in organisms or transfer

functions from an organism to another. This field is controlled by technical specifications to avoid GMO spreading (for example).

## 5 - Events cards (16 cards)

### Transplanting (5 cards)



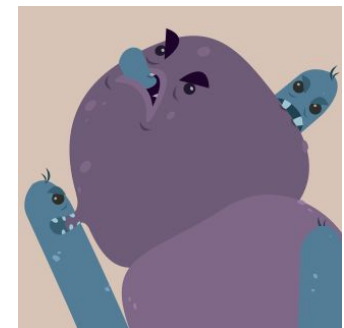
**Distinctive feature:** Growth medium changes! Turn the first card of the media stack, it becomes the growth medium for all the bacteria. Play this card only at your turn.

*Learn more:* in the laboratory, transplanting means taking a bacterial colony and putting it on another growth medium.

### Bacillus vs. Coccus (1 card)

**Distinctive feature:** once this card is played, the bacillus team confronts the coccus team. You must play this card as soon as you draw it, it is discarded when one of the teams wins.

*Learn more:* bacillus and coccus are different types of bacteria, respectively rod and round-shaped. This card was mostly designed to show the diversity of shapes but bacteria do not naturally have affinity according to their shape.



## Medical treatment (1 card)



**Distinctive feature:** all human pathogen colonies loses 2 log. Discard after use.

**Learn more:** in case of wound or infection, antiseptics and antibiotics are used to protect or treat the organism. These agents reduce the bacterial proliferation but the infection can start again when the treatment is stopped. This relapse especially occurs when the immune

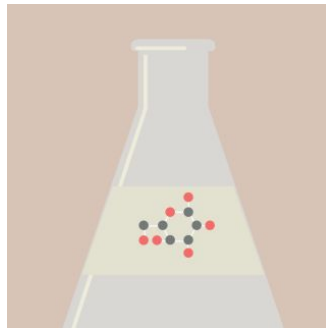
system is weak.

## Glucose, Galactose, Xylose, Fructose (1 card of each)

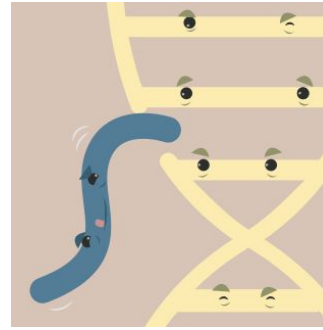
**Distinctive feature:** during your next division you will win 1 extra log (+ 2 log). Discard this card after use.

**Learn more:** fructose, galactose and xylose provide additional carbon source to the colony, improving its division. Are very common sugars in nature.

Glucose is one of the most efficient sugars to support the bacterial growth. To optimize its consumption, bacteria often have systems of regulation to consume glucose before any other carbon source.



## Transposon (3 cards)



**Distinctive feature:** you can play the transposon on the plasmid of one of your opponents, it is immediately discarded.

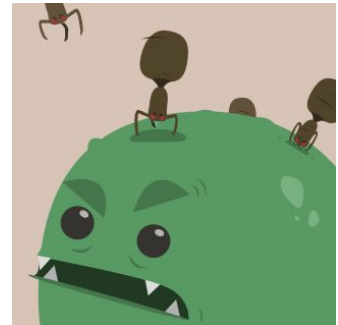
**Learn more:** transposable elements are DNA sequences that can change their position within a genome. They can extract themselves and insert in another locus (in a chromosomal DNA or a plasmid). Their integration can induce a loss of gene function. They exist in all

living organisms and are powerful evolution trigger.

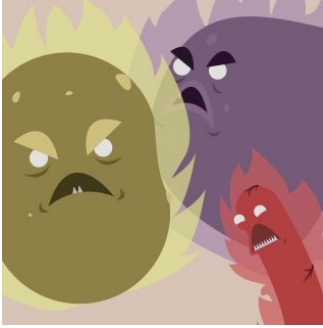
## Phage attack (1 card)

**Distinctive feature:** choose the direction in which the phage is spreading. During the attack you lose 1 log, your neighbour 2 and his neighbour 3.

**Learn more:** phages are like viruses that target bacteria. They are only composed of a protein structure that contains their DNA and allows them to anchor on their bacterial prey. Their DNA is then injected inside the bacterium where they replicate, thus producing new phages. This mechanism can result in the cell lysis (cell death), hence in the release of newly created phages in the environment, leading to the infection of further bacteria.



## Immunosuppression (1 card)



**Distinctive feature:** all human pathogens win + 2 log. Discard after use.

*Learn more:* pathogen bacteria are often commensal, meaning that they are living on or in their host but they do not trigger symptoms. An equilibrium is reached between the bacteria and the immune system of the host. A weakening of this immune system can lead to the thriving of the bacteria and the installation of the pathology.