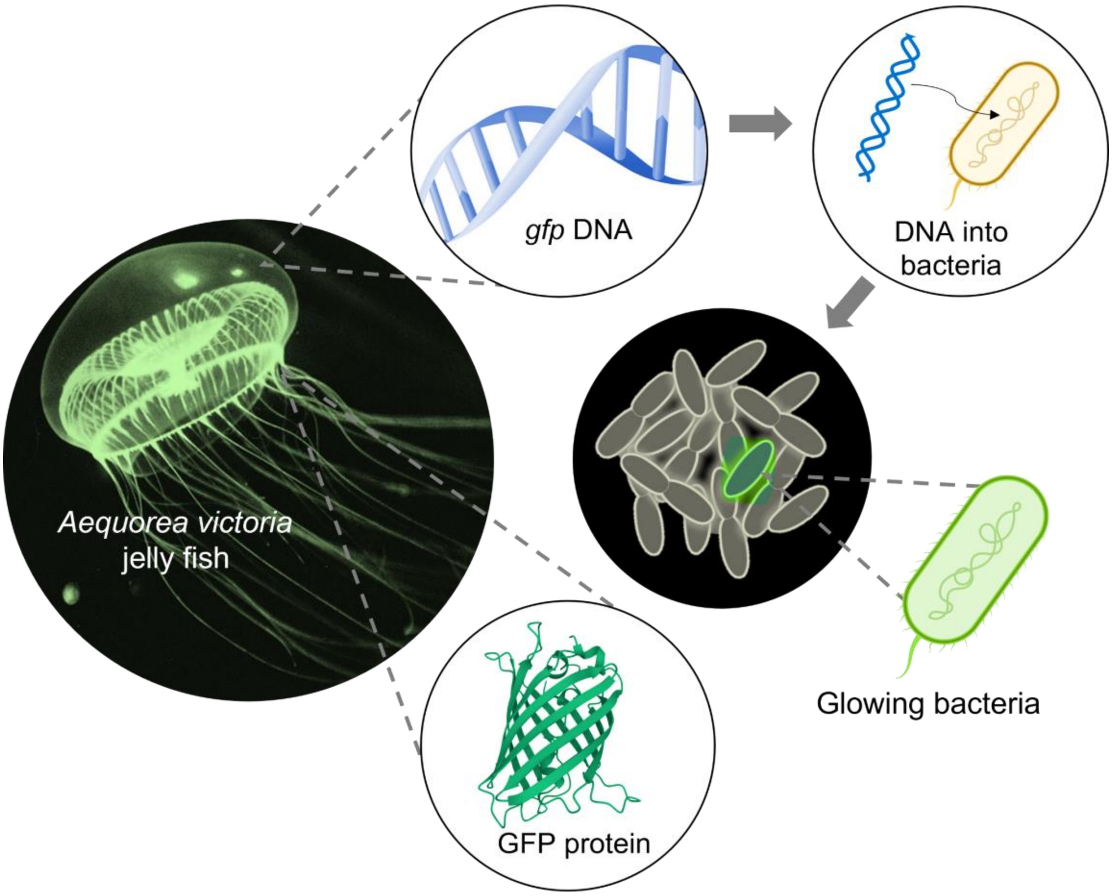
Study tools: glowing and colourful proteins as visualisation aids

Granddad, is it true that scientists make bacteria glow by using spare parts from a jellyfish?



Green fluorescent protein (GFP) originates from a jellyfish. Originally, the GFP protein was purified from the jellyfish *Aequorea victoria*. Later, the DNA sequence of the GFP protein was determined by scientists and the ability to produce GFP was transferred to bacteria. When producing a glowing protein such as GFP, the bacteria would fluoresce green and, in the presence of other microbes, be easily recognised and distinguished. Photo of *Aequorea victoria* by Jonathan Diemel. The GFP protein crystal structure 1EMA from the Protein Data Bank (PDB).

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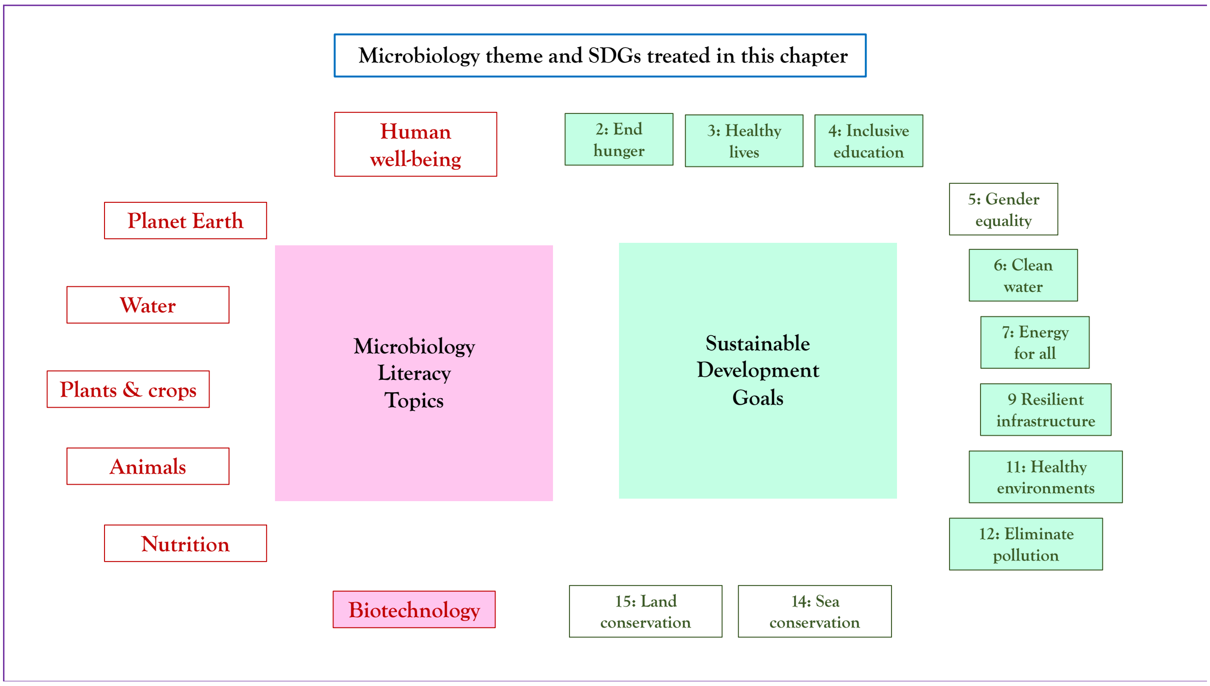
Glowing and colourful proteins as visualisation aids

Storyline

Proteins serve some of the most important functions in living organisms but are small and difficult to study. Fortunately, some proteins glow or make colourful compounds and are therefore easy to see. Proteins with properties such as fluorescence or luminescence are found in many organisms in nature and have been used in microbiology research for decades. These *reporter proteins* can be grafted on to other proteins of interest to help reveal the details of microorganisms and molecular biology, and to enable the design of biosensors for detecting polluting chemicals or diagnosing disease.

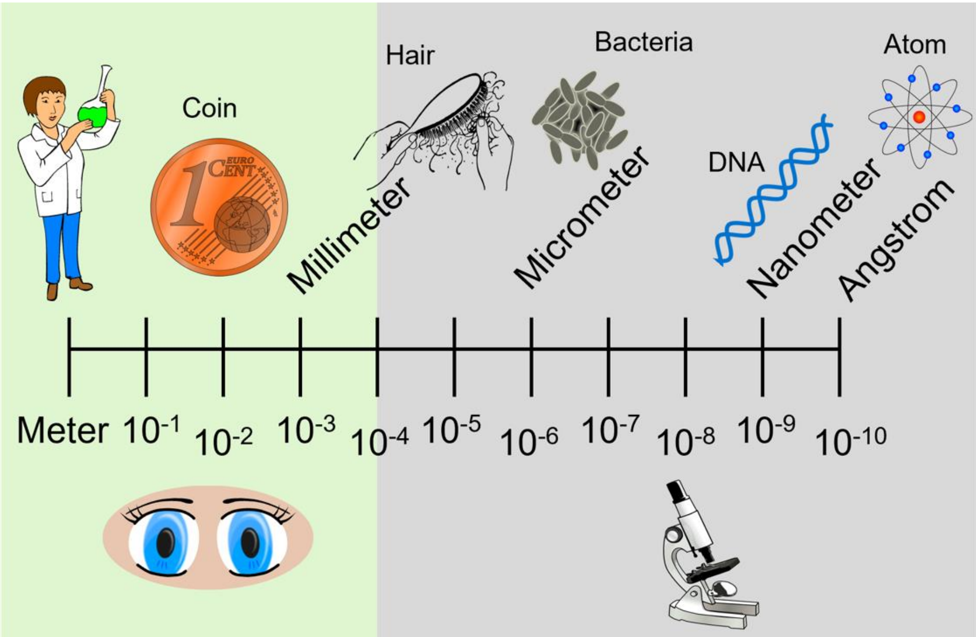
The Microbiology and Societal Context

The microbiology: Native reporter proteins; the history of microbiology; study tools; visualization of molecular interactions; applications of reporter proteins in basic research; biosensors; future applications of reporter proteins in biotechnology. *Sustainability issues:* Hunger; health; education; clean water; clean energy; innovation; sustainable communities; responsible production.



The importance of reporter proteins in microbiology

1. ***How do scientists work with microscopic organisms like bacteria when they cannot see them?*** Studying microorganisms on the micrometre scale is not easy. Microorganisms and bacteria are very small - they are *micro*. The average microbe is a million times smaller than a human being. To view the microworld you need to use very strong glasses or instruments like a microscope. But when you visualize different microorganisms, they often look the same. With the help of glowing molecules such as the green fluorescent protein (GFP), we can mark our bacteria, enabling us to distinguish one specific type or species from another.



The length scale of biology. An item becomes 10 times smaller for every step down starting at one meter. The green part of the picture represents items that are naked to the eye whereas items in the grey part require a microscope for visualization. Bacteria and microorganisms are in the micro-scale.

2. ***The story of the green fluorescent protein.*** The story of GFP is a beautiful example of a curiosity-driven, basic and simple scientific question - *what makes jellyfish glow?* – and how pursuing the answer with many different approaches caused a dramatic paradigm shift in science.

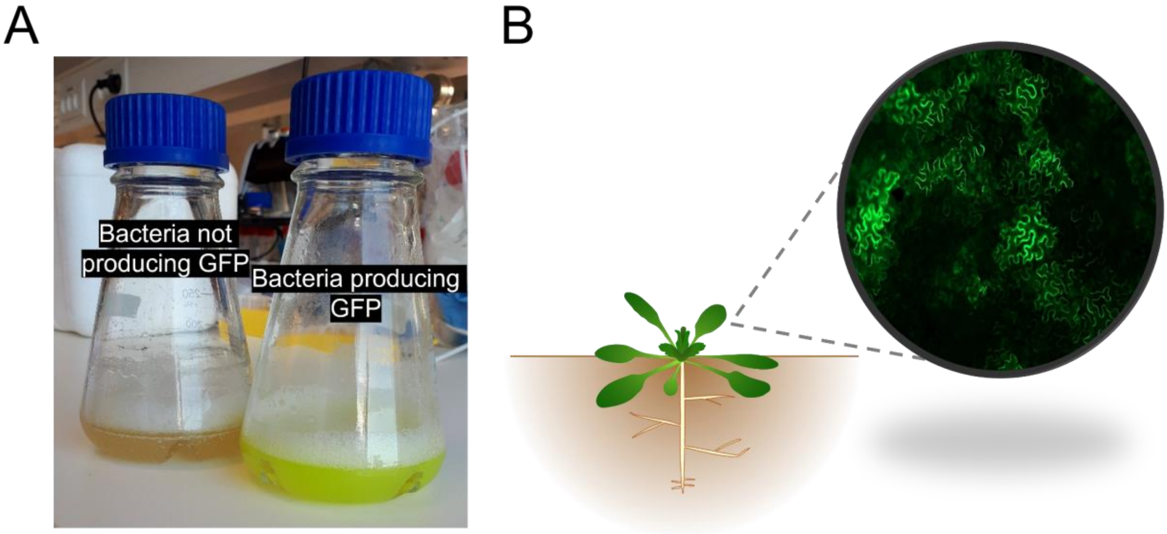
GFP was discovered by Osamu Shimomura in the early 1960s. Shimomura would for years go on fishing expedition trips with his family to catch thousands of jellyfish, in order to have sufficient material to be able to isolate and purify enough of the fluorescent material to study the molecules responsible for the green glow.

The story of GFP also has a scientific "eureka moment" (the story goes that the ancient Greek scientist Archimedes shouted "Eureka! Eureka!" after he had stepped into water, making him realize the scientific principle that the volume of water displaced is equal to the volume of a submerged object). Similarly, after the gene coding for the 'green glow' protein – now called green fluorescent protein - had been discovered, named *gfp* and its sequence determined, Martin Chalfie transferred it into both bacteria and worms, which was enough to make these highly different organisms glow brightly green – Eureka!

In the following years, Roger Tsien led research efforts that would considerably improve the properties of GFP to make it a universal research tool. For example, they also made red fluorescent proteins. Together, these developments were recognized by the Nobel prize in chemistry in 2008, because GFP completely changed how we can study microscopic phenomena.

3. ***Bacteria are micro and the molecules within them are a thousand times smaller.*** To make bacteria produce glowing proteins we need to give them the genetic ability by providing the DNA that encodes the glowing protein. DNA is the molecular recipe for everything living and can easily be cut and assembled in new ways with genetic scissors and glue in the molecular biology laboratory – like LEGO bricks.

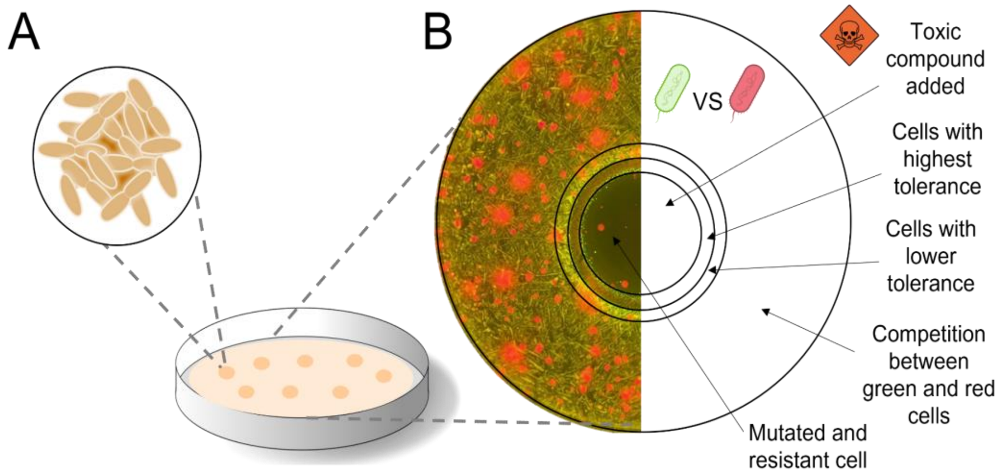
Besides marking bacteria, we can monitor and observe when growth is happening – when one microbe becomes two and eventually turn into millions. And we can study a new protein with unknown function if we fuse it with a glowing protein and follow when it is made and how it moves within the compartments and spaces of a living cell. We can even use glowing proteins like GFP in bigger organisms and for example make partial or complete fluorescent plants.



Glowing proteins help visualizing biology. A) When millions are grown together in a culture in the laboratory it is possible to see if bacteria are producing GFP or not. Photo by Pernille Ott Frendorf. **B)** By only targeting the production of GFP to the leaf cells of a plant in the laboratory, fluorescent jigsaw-puzzle patterns can be observed. Photo by Morten H.H. Nørholm.

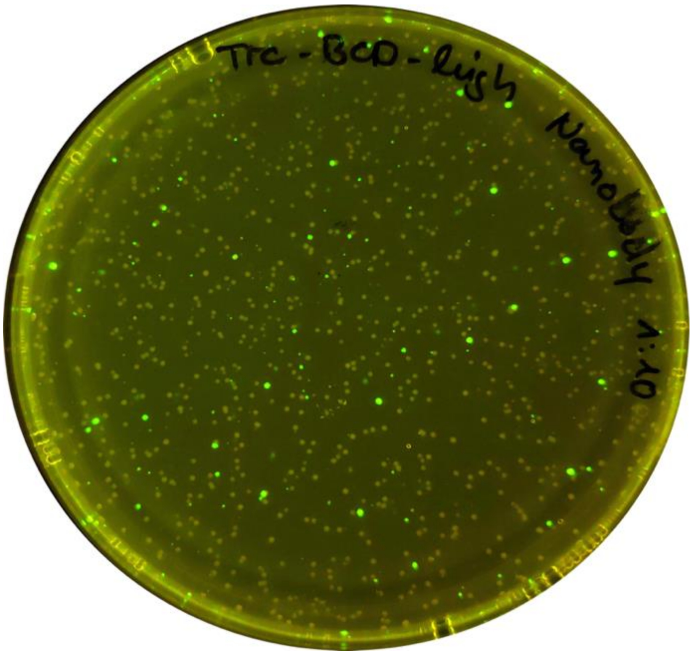
4. ***How do fluorescence reporters improve our lives?*** Bacteria live very tightly together with their neighbours in so-called "colonies" made of billions of bacteria that together are visible to the naked eye. When studying bacteria in the laboratory, these colonies are formed on gel-like agar media in small round petri dishes that contain nutrients such as sugars, vitamins, and minerals. As for many other animals living in the same environment in nature, bacteria compete for available nutrients. Bacteria have different skills and talents like us humans and therefore often one type or group of bacteria is often better suited and adapted to live in a specific environment than others. By growing bacteria with different nutrients or toxic chemicals in petri dishes it is possible to study the competition between different strains of bacteria by making them produce glowing proteins in different colors such as green and red with the glowing proteins GFP and red fluorescent protein (RFP).

Two different strains of bacteria can be spread in an equal number on an agar plate and a drop of a toxic compound can be added in the middle. By observing the growth close to the place where the toxic compound is added, we can monitor the tolerance: if the green bacteria grow closer to the drop, they have higher tolerance than the red ones. Investigating tolerances to compounds can be useful when searching for new antibiotics to combat bad and pathogenic bacteria that infect humans and makes us sick. Bacteria tolerant of nasty chemicals can also be helpful to us - for example by cleaning up pollution in the environment.



Competition between two types of bacteria on agar plates. A) In the laboratory bacteria are grown in agar plates in petri dishes. They form colonies where millions of bacteria live tightly together. They are genetically the same. **B)** Adding a toxic compound to an agar plate covered with equal amounts of two bacteria it is possible to compete them with each other and investigate the tolerance to the compound. The cells will grow according to their tolerance to the compound. In the middle genetically changed (mutated) "escapers" can occur that are resistant to the toxic compound. Photo by Ida Lauritsen.

Glowing bacteria can also help us identify bacteria that have been created for use in biotechnology and biomedicine. One of the simplest methods for making microbial cell factories able to produce proteins we need is based on green-glowing bacteria that turn white when modified with new DNA. The reason is that the gene for the protein we need is inserted into the middle of the *gfp* gene, thereby interrupting its sequence and inactivating its ability to direct the production of GFP, a process termed *insertional inactivation*. With this approach, researchers only have to mix bacteria with DNA – for example encoding a pharmaceutical like insulin – and pick the white colonies among a background of normal fluorescent green colonies that appear on agar plates the next day. Such bacteria are now able to produce insulin.

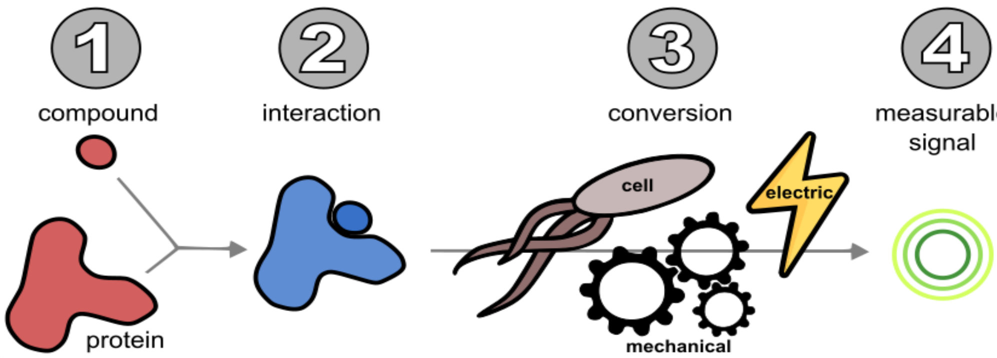


Identification of bacterial colonies with a modified gene content. On this agar plate, bacterial colonies fluoresce green unless their DNA has been changed. This way, bacteria with new DNA inserted, such as a gene encoding insulin, are easily recognized. Photo by Anja Ehrmann and Carolyn Bayer.

5. ***Sensing cells contribute to a more sustainable world.*** The ability of microorganisms to sense compounds in the environment is useful - not just for the discovery of new compounds, but also for detection of chemicals or other microorganisms. Devices derived from biological systems that sense such conditions are called biosensors, and they convert a biological interaction, e.g., between proteins and chemicals, to a measurable signal using study tools such as reporter proteins.

Most biosensors can be assigned to one of two groups; those that use only spare parts from microorganisms, such as DNA or proteins, and those that use a living cell with its own DNA and proteins. Without a cell, a biosensor requires help from us humans to convert a molecular interaction into a signal. These convertors can for example be an electric component, which converts the signal, or an enzymatic dye that only appears when the right protein binds, such as we know it from rapid COVID-19 or supermarket pregnancy tests. While these produce plastic waste, they are assisting the health care systems and saving resources that would otherwise be spent on doctors and hospitals.

When using cell biosensors, the cell itself converts the biological activity targeted into a detectable signal. These signals can for example be the β-galactosidase enzyme that can produce a blue coloured dye, the glow of GFP or light produced by the bioluminescent luciferase enzyme. These reporter proteins tell us what is going on inside the cell, and they can be designed to report on many different events, decided by the scientist that made them. By carefully choosing combinations of DNA sequences, a scientist can mix-and-match between what to detect (the *sensing* part) and how to report it (the *signal* part). This enables scientists to make microorganisms that detect environmental pollution, help diagnose sick people, and ensure good food quality.



(1) Biosensors sense specific molecules, for example chemicals in the environment, or proteins that cause disease. (2) Some biological molecules such as proteins interact with other molecules or can be designed to do so. (3) In order for such an interaction to be observed by humans, it needs conversion from microscopic interactions to a measurable signal. Mechanical biosensors are passive and give a response such as by colour. Electric biosensors convert interactions to electric signals by using the physical properties of molecules. Cellular biosensors use the cell and its own spare parts to create a signal. (4) In the end, the signal can be measured. Some signals, such electricity or light, are converted to a number so that the scientists can measure its strength, and other signals, such as colour, may be strong enough for the scientists to see the result with their own eyes.

6. ***Will glowing proteins one day replace streetlights?*** Some reporter proteins have direct practical applications, specifically those that produce light (luminescence), such as the luciferase enzyme. Luminescent abilities (bioluminescence) are found in many organisms in nature, from single-celled algae in the ocean, to fireflies in the air, and fungi growing from the forest floor. The light-emitting abilities of algae has been used to create algae lamps, providing alternative night light from a flask when shaken. Some of these light-emitting abilities have also been

transferred to bacteria, creating beautiful art pieces, and to desk plants, creating natural desk lamps.

This enables thought experiments on the future of lightning and our dependence on electricity and fossil fuels: using algae, scientists have created streetlamps that do not need electricity and even absorb carbon dioxide from the air using photosynthesis. With what we have learned from algae and engineered bioluminescent plants, it has become theoretically possible to create street lighting using bioluminescent trees, and recent research indicates that bioluminescence from fungi could make for even stronger glowing plants.

However, although these technologies are exciting and may provide a carbon dioxide-free alternative to lighting, it is always important to consider the consequences of such actions. Since bioluminescence would become part of the engineered organisms providing the light, we would need to figure out how the lighting can be turned on and off as for electricity. The light from traditional, electric streetlamps, which are sometimes turned off during the quiet hours of the day, have been shown to disturb the daily rhythms of birds. These consequences might worsen using constant street lighting that cannot be turned off, with unforeseen consequences for the affected ecosystems. Thus: before exploiting biological lighting on our streets, we need to figure out how to turn it off and on.

Relevance for Sustainable Development Goals and Grand Challenges

The use of study tools such as reporter proteins relates to several SDGs, including:

- **Goal 2: End hunger, achieve food security and improved nutrition and promote sustainable agriculture.** The use of study tools in biosensors and assays provides opportunity for detecting contaminants and pollution in food supplies.
- **Goal 3: Ensure healthy lives and promote well-being for all at all ages.** Study tools contribute to innovative technologies within healthcare and diagnostics.
- **Goal 4: Ensure inclusive and equitable quality education and promote lifelong learning opportunities for all.** Study tools were developed from native reporter proteins to ease certain aspects of research and still in this regard contribute substantially to research efforts. Furthermore, they are also included in many educational experimental kits with the purpose of making microbiology more approachable and entertaining for school-age children and teenagers.
- **Goal 6: Ensure availability and sustainable management of water and sanitation for all.** As for food, the use of study tools in biosensors and assays provides opportunity for detecting contaminations and pollution in water supplies.
- **Goal 7: Ensure access to affordable, reliable, sustainable, and modern energy for all.** The native reporter proteins are being exploited in microbiological contexts for the purpose of generating microbial, sustainable lighting that does not depend on electricity.
- **Goal 9: Build resilient infrastructure, promote inclusive and sustainable industrialization and foster innovation.** The use of study tools in research and their exploitations in technology development contributes to innovation of technologies within the life science and health care industries.
- **Goal 11: Make cities and human settlements inclusive, safe, resilient, and sustainable.** Reporter proteins have native functions enabling them to be applied in functions required for society, such as lighting.
- **Goal 12: Ensure sustainable consumption and production patterns.** The use of reporter proteins and biomarkers in bioproduction enhances quality control and minimizes waste.

Potential Implications for Decisions

1. ***Individual***
   a. Using fluorescent reporter proteins to help diagnose sick people and ensure good food quality
   b. "Cut-and-paste" the building blocks together to make a living organism

2. ***Community policies***
   a. The cost of research could be used in other sectors
   b. Proper disposal of GMO waste, no environmental pollution to e.g. water

3. ***Global policies***
   a. Regulations on GMO
   b. Reduce emission of greenhouse gases by using biosensors as streetlights
   c. Using protein reporters for competition assays that can facilitate the identification of new antibiotics for treating infections
   d. Reporter proteins in the improvement of DNA assembly technologies for production of medicine such as insulin

Pupil participation

1. ***Class discussions***
   a. Disadvantages and advantages of using study tools such as GFP
   b. Are we playing "god" when we remove traits and give new abilities to living organisms?
   c. Is it really cleaner energy that is developed in research when we are using one-time use plasticware in laboratories?
   d. Are there other features or traits that can be taken from nature and applied in a microorganism for the human benefit?

2. ***Pupil stakeholder awareness***
   a. Are there any drawbacks of using study tools/fluorescent proteins for research purposes?
   b. Can you find other examples where you use a colour as an indicator of something? Or to mark and detect something? And why is it smart that the indicator is a living organism?
   c. Can we use microorganisms for our benefit?

3. ***Exercises***
   a. Design your own biosensor system

The evidence base, further reading and teaching aids

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Glossary

Agar: a jelly-like substance consisting of polysaccharides obtained from algae that makes it easy to grow and handle bacteria
Luminescence: spontaneous emission of light by a substance not resulting from heat
Fluorescence: a form of luminescence where emission of light by a substance is induced by absorption of light or other electromagnetic radiation
Green fluorescent protein (GFP): refers to specific protein from the jellyfish Aequorea victoria that fluoresces in the green spectrum of light
Red fluorescent protein (RFP): refers to a protein that fluoresces in the red spectrum of light
Bacterial colonies: billions of bacteria that form small structures on agar plates that are visible to the naked eye

Bacterial escapers: individuals in a bacterial population that have gained a new ability to grow better.
Biosensor: a device based directly on living cells or of biological origin that is designed to sense specific chemical clues such as pollution or disease-related molecules