# iGEM Activity for Biofutures 2011

Tutor version

UQ-Australia iGEM Team
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# Tutor Instructions

This activity is intended to follow a presentation introducing synthetic biology and iGEM.
For the activity,

- The students (80) will be split into 8 groups of 10 with 2 tutors (already determined by Biofutures).
- Each group will be given one scenario.
- Tutors will be familiar/ have notes on the past team projects.
- The group will have butchers paper and marker to record/ illustrate their discussion.

Students will

1. Come up with a solution to the scenario - Tutors will guide/ lead them towards the past team
2. Discuss advantages/ disadvantages, limitations, feasibility
3. Other applications of their system
4. Other solutions to the scenario
5. Record their discussion on butcher's paper

From this activity, students should

- Go through some of the thinking process followed by the iGEM students when formulating a project
- Have an idea of what has been for synthetic biology/ iGEM
- Put synthetic biology into perspective

NOTE: the discussion for the activity will be (perhaps heavily) lead by the tutors.

TUTORS MUST HAVE AN IDEA THE ASSOCIATED PAST iGEM TEAM PROJECT!

There is no need for the tutor to just tell the students what happened, nor is there a need for them to know *exactly* what the past team has done. We really would just like the students to have an idea of what has been done, but more importantly, we would like them to discuss this amongst themselves and to think about the consequences themselves. If however, there is that awkward silence, then tutors should step in and throw an idea at them. There are notes accompanying this, in case you too are strapped for ideas.

Note that we have kept your notes reasonably brief. There is no need to explain to them plasmid goes to gene goes to protein etc. This is why we have the BioBrick model. We want interchangeable standardised parts where we really can just something from one thing and put it somewhere else. Of course, it would be beneficial, particularly if asked, to mention inhomogeneities, nonlinearities etc. that would make the BioBrick model a little more complex than that.

## Student Instructions

For the activity,

- The students (80) will be split into 8 groups of 10 with 2 tutors (already determined by Biofutures).
- Each group will be given one scenario.
- Tutors will be familiar/ have notes on the past team projects.
- The group will have butchers paper and marker to record/ illustrate their discussion.

Yes, this is just another butcher's paper activity.
Students will

1. Come up with a solution to the scenario - Tutors will guide/ lead them towards the past team
2. Discuss advantages/ disadvantages, limitations, feasibility
3. Other applications of their system
4. Other solutions to the scenario
5. Record their discussion on butcher's paper

Don't worry if you are strapped for ideas. The tutors will guide your discussion, but it is your discussion. You will find that your scenario is a real scenario and has motivated research in that area. You will find that your solution is probably like one that has been implemented by a past iGEM project. If it isn't, then you've got a project ready for your team to pursue!

Note: there is are no right or wrong answers. There are no answers. The idea of this activity is for you to practice your thinking, and communication skills.

# Scenario A (Heavy metal biosensor and bioprecipitation)

Heavy metals are dangerous contaminants in water supplies. This is a significant problem in developed and developing counties. We may recall, Mt Isa, a mining town in North Queensland recently had an issue with mercury contaminating their water supplies. Test for contaminants, particularly heavy metals such as mercury, is performed with chemical testing kits. This is both costly and may be of unsatisfactory accuracy. Filtration of these heavy metals from water is often both costly and intensive.

- Applying the concept of synthetic biology, what possible solutions are there in order to tackle this problem?
- Also, when constructing the possible solution, think about its
  - advantages/disadvantages
  - limitations
  - feasibility.
- What other backup solutions do you have?
- Hints:
  - Synthetic biology techniques uses interchangeable standardised genetic parts
  - How do we find the mercury?
  - How do we remove the mercury?

## Past Team Solution

Investigated by Peking 2010, UQ Australia 2009 and MIT 2007 iGEM Teams.

1. There exists a family of proteins that control metal ion, radical, and small organic molecule concentrations inside bacterial cells. Some of these can respond to Cd(2+), Zn(2+), Co(2+), Cu(+), Ag(+), Au(+), Hg(2+), and Pb(2+).
2. A response to these can be signalled by bacteria carrying these proteins, e.g. by fluorescing

1. Many bacteria have resistance to heavy metals. E.g. *Cupriavidis* metallidurans has genes resistant to Ag(I), Cd(II), Co(II), Cr(IV), Hg(II), Ni(II), Pb(II), and Zn(II).
2. Bacteria carrying this resistance can then have the heavy metal bind to their genetic circuit

- Advantages:
  - Portable
  - Bacteria safer than heavy metals?
  - Not introducing, perhaps harsher, chemicals to clean the contaminants
- Disadvantages:
  - We will have bacteria in water supplies
  - Is it scalable?
  - How would you collect the mercury-filled bacteria?
  - If our test for detecting contaminants results in a negative, then we've just release bacteria into a clean environment
- Further uses:
  - Could generalise to other contaminants, e.g. other heavy metals
  - We have biosensor - what else can we find?
- Other solutions:
  - Reduce mercury entering the system
  - Safer water storage technologies

## Scenario B (Bacteria sensing nitrates)

Many crop types need to be harvested and replanted from scratch on a seasonal basis. To maintain nutrient levels in the soil, farmers often have to put down considerable quantities of fertiliser. This is costly, and a fair proportion of it ends up landing on soil that has enough nutrients anyway. Additionally, some of the nutrients in the fertiliser are often washed away, affecting local ecosystems. This process is called eutrophication. This is a major environmental concern, as it can cause algal blooms that drain oxygen out of rivers and lakes, killing fish and other wildlife. Fertiliser production is also a significant contributor to global greenhouse emissions. Reducing excess fertiliser use would be of great benefit to both food producers and the environment.

- Applying the concept of synthetic biology, what possible solutions are there in order to tackle this problem?
- Also, when constructing the possible solution, think about its
  - advantages/disadvantages
  - limitations
  - feasibility.
- What other backup solutions do you have?
- Hints:
  - Synthetic biology techniques uses interchangeable standardised genetic parts
  - Fertilising soil is primarily to increase the nitrate levels in soil
  - How do know if we have enough nitrates in the soil?

### Past Team Solution

Investigated by BCCS Bristol 2010 iGEM Team.

They have made beads of bacteria which change colour in the presence of nitrates.

1. There exists a family of proteins that respond to nitrate
2. Bacteria was set to constitutively fluoresce red
3. In the presence of nitrate, bacteria would fluoresce green
4. To see this fluorescence, UV light would be needed

- Advantages:
  - Cheap - only fertilising what is needed to be fertilised
  - Bacteria contained in beads - easy to distribute
- Disadvantages:
  - We will have bacteria in our crops
  - Is it scalable?
  - Will need UV to see the colour of fluorescence?
  - Will nitrate levels change from measuring the nitrate level to planting crops?
- Further uses:
  - Could generalise to other nutrients/ properties e.g. pH?
  - We have biosensor - what else can we find?
- Other solutions:
  - Legumes store nitrates
  - Companion planting

# Scenario C (Oil sensing and degrading bacteria)

Pollution of soil and water environments by crude oil has been, and is still today, an important environmental issue. Crude oil is a complex mixture of thousands of compounds, of which alkanes constitute the major fraction. Although they are chemically relatively inert, several micro-organisms can efficiently degrade most of them. Literature research has revealed a number of genes that are most likely responsible for this degradation.

- Applying the concept of synthetic biology, what possible solutions are there in order to tackle this problem?
- Also, when constructing the possible solution, think about its
  - advantages/disadvantages
  - limitations
  - feasibility.
- What other backup solutions do you have?
- Hints:
  1. Synthetic biology techniques uses interchangeable standardised genetic parts
  2. We need to degrade the oil
  3. We need to find/ identify the oil
  4. Suppose an oil spill occurred in the ocean. How will bacteria survive?
  5. Can this all dissolve in water?

## Past Team Solution

Investigated by TU Delft 2010 iGEM Team.

They have designed a system that is able to detect and degrade alkanes and survive in seawater. Note that this was presented as separate mini-projects, not one giant super-bacteria.

Note: I don't think they had everything working experimentally.

1. There exists some genes which are believed to be responsible for the ability of some microorganisms to be able to degrade alkanes.
2. Some genes are controlled by alkane levels. These can be combined with fluorescence to identify the alkane.
3. There was an attempt to use the alkane as the carbon and energy source. Note the challenge: salinity and organic solvent toxicity.
4. There exists some genes which are believed to be responsible for the ability of some microorganisms to be able to produce biosurfactants.

- Advantages:
  - Easy to transport
  - Not introducing, perhaps harsher, chemicals to clean the contaminants
- Disadvantages:
  - There were multiple bacteria produced
  - Is it scalable?
  - How would you collect the environment-resistant oil-filled bacteria? Will it all just dissolve?
- Further uses:
  - Could generalise to other contaminants, e.g. alkenes (another component of crude oil)
  - We have biosensor - what else can we find?
- Other solutions:
  - Further research into oil distribution networks
  - Safer oil storage and transportation technologies

## Scenario D (Cyanobacteria producing ethanol)

With the limited fossil fuel resources, society is going to have an energy crisis. There is an ongoing push for alternative energies to lessen the human impact on the environment. The main renewable source of energy is the sun. Plants use the sun to convert sunlight to energy through the process of photosynthesis. There are other investigated alternatives such as biofuels, such as ethanol. Ethanol has been used for many decades so we already have the technology to use it. Likewise with butanol, which has advantages over ethanol in terms of the energy density, safety and adaptability. However, present sources for biofuels are in direct conflict with food production.

- Applying the concept of synthetic biology, what possible solutions are there in order to tackle this problem?
- Also, when constructing the possible solution, think about its
  - advantages/disadvantages
  - limitations
  - feasibility.
- What other backup solutions do you have?
- Hints:
  - Synthetic biology techniques uses interchangeable standardised genetic parts
  - Cyanobacteria photosynthesise
  - There exists ethanol-producing bacteria

### Past Team Solution

Investigated by Uppsala 2009 iGEM Team.

They have used two genes encoding enzymes from ethanol producing bacteria and cloned them into photosynthetic cyanobacteria (blue-green algae). The enzymes convert the photosynthesis products into ethanol. Further idea was to produce butanol instead of ethanol.

NOTE: Ethanol is inferior to fossil fuels with respect to energy density, corrosiveness, safety. So perhaps try butanol production instead of ethanol production (not carried through with any teams)

- Advantages:
  - not competing with food production
  - Low cost
  - Portable
  - Fast adaptation to existing infrastructure/ technology
- Disadvantages:
  - GM bacteria lives in labs
  - Is it scalable?
  - How would you 'harvest' the ethanol?
- Further uses:
  - Bacteria to take sunlight into ethanol.
  - Why not carbon dioxide into ethanol/ butanol?
  - Why not methane into ethanol/ butanol?
- Other solutions:
  - Using less power?
  - Wind/ geothermal/ tidal
  - Solar ? organic (i.e. carbon-based) solar cells
  - More efficient technologies - more efficient power distribution

# Scenario E (Bacteria to induce rainfall)

We are familiar with the concept of saving water. The past few years had water restrictions, droughts, but also floods. Although our freshwater supply is still dependent on rainfall, in urban areas, we have tried to resolve this issue with the building of dams to store water for times when we have low rainfall. For sparsely populated areas however, this becomes very costly and impractical, on the scale as for done with Brisbane with Wivenhoe and Somerset. The arid Australian climate causes widespread problems for the agricultural industries with the scarcity of water hindering the production of crops and livestock.

- Applying the concept of synthetic biology, what possible solutions are there in order to tackle this problem?
- Also, when constructing the possible solution, think about its
  - advantages/disadvantages
  - limitations
  - feasibility.
- What other backup solutions do you have?
- Hints:
  - Synthetic biology techniques uses interchangeable standardised genetic parts
  - P. syringae (bacteria) is a ubiquitous airborne bacterium which expresses a protein for ice nucleation, including precipitation. However, optimal growth of P. syringae occurs at 295K.

## Past Team Solution

Investigated by UQ Australia 2009 iGEM Team.

1. So we have a (naturally occurring) bacteria that has the ability to induce rain. However, this bacteria lives in colder climates whist the climate in Australia is hot.
2. UQ Australia 2009 have increased the temperature tolerance of this bacteria so that it can live in hotter climates.
3. This was done by introducing five heat-shock genes to the bacteria so that it would be more tolerant of the temperature.

- Advantages:
  - This is a naturally occurring bacteria which is already present in most (cold) environments
  - Has the ability to induce (perhaps control) rain, important resource
  - Cloud seeding, with bacteria, already used in colder environments, eg ski resorts, to induce ice/snow formation
  - Simple to implement, just release the bacteria
  - Scalable?
- Disadvantages:
  - GM bacteria live in the lab
  - Unknown risks wrt releasing a stronger organism into a new environment
  - Ice nucleation bacteria are a pathogen to plants ? causing them to freeze, how will the presence of more freezing bacteria affect horticulture?
- Further uses:
  - Generalises to any hot arid environment, eg Africa
  - Removal of such bacteria could reduce rainfall during flooding seasons
  - Snow/ ice in the desert?
- Other solutions:
  - Further research into water distribution networks, is long-distance piping practical?
  - Further study into dams/lakes/waterway flows ? minimising evaporation
  - Recycling water

# Scenario F (Cheap and feasible artificial lights using a biological system)

Lighting (street lights, billboards, etc.) accounts for approximately 10 % of electricity use in Britain. Considering the energy crisis we have and the world's desperate need for alternative source of energy, there is a need for lighting system that does not make use of our depleting source of energy. When we consider lighting, remember that TV screens, computer monitors and your phone screen uses lights.

- Applying the concept of synthetic biology, what possible solutions are there in order to tackle this problem?
- Also, when constructing the possible solution, think about its
  - advantages/disadvantages
  - limitations
  - feasibility.
- What other backup solutions do you have?

## Past Team Solution

Investigated by Cambridge 2010 and Valencia 2009 iGEM Teams.

Knowing that there are organisms that can emit light by themselves, they isolated the genes responsible for emission of light (luciferase) and put the gene into the bacterial cell such that bacterial cells can emit light (bioluminescent bacteria).

- Why use bacterial cells instead of just having light emitting fireflies? The reason behind this is that bacterial cells' genes are easier to manipulate and large number of bacterial cells can easily be produced. Also, bacterial cells can be used to infect other organisms so that they can emit light as well)

Using the bioluminescent bacterial cells, lighting system can be established where it can be used as a street light, emergency exit signs or even reading lamps. They even went further to think of having bioluminescent trees whereby the trees are infected with bioluminescent bacteria. These light emitting trees can then be planted along the streets. This not only gives possibility of eliminating air pollution, but also it can get rid of street lights.

- Advantages:
  - It can effectively reduce the energy usage for lighting.
  - It is cost effective (cheap and easy to be produced). It can be a cheap alternative to current lighting system.
  - It has many applications. Genes responsible for emission of lights can be manipulated to produce different colours and different intensity of light.
- Disadvantages:
  - Since bacterial cells are living organisms, effort must be put into keeping them alive (giving them nutrients, having them in an optimal environment ? temperature, humidity, etc.-).
  - There are health concerns. Having bioluminescent bacterial cells as a lighting system would mean that humans would have bacterial cells near them. Although bacterial cells that are not hazardous would be used, this would still pose significant health threat to society.
- Limitations:
  - Intensity of light emitted from bacterial cells would not be as strong as light produced by electricity. This would limit the scope of applications of bioluminescent bacteria.
  - There exists (Centre for Organic Photonics and Electronics) research in the development of organic (carbon-based) light emitting diode (OLEDs)

# Scenario G **(Usage of bacteria to mend the leaky pipe)**

Having a leaky pipe can be troublesome, for it not only causes the loss of billions of litres of water, but also having a leak in sewage or oil pipe can be damaging to the environment as well. Some of us might recall the recent BP spill in the Gulf of Mexico. There are occasions where the leak in the pipe is big enough so that it can be spotted easily and thus, make the mending easy. However, it is nearly impossible to locate all the microscopic leaks in the pipe that equally cause loss in revenues and environmental damage.

- Applying the concept of synthetic biology, what possible solutions are there in order to tackle this problem?
- Also, when constructing the possible solution, think about its
  - advantages/disadvantages
  - limitations
  - feasibility.
- What other backup solutions do you have?

## Past Team Solution

Investigated by Alberdeen Scotland 2009 iGEM Team.

They made use of one of the characteristics of bacteria, chemotaxis. Chemotaxis is the directed movement of bacterial cells towards the attractant or away from repellent. They were able to make the bacterial cells to move towards the leak in the pipe by making the pipe to release an attractant at the damaged/corroded regions. Also, using synthetic biology, they manipulated bacterial genes such that they would produce glue-like substances that can mend the damaged pipe. Since it would be problematic to release glue-like substances before reaching the damaged site, they would be triggered to produce glue-like substances only when they sense that other bacterial cells are close to themselves (mechanism: quorum sensing). Genes would be manipulated to make the bacterial cell to undergo autolysis ("bursting") after a certain time, in order to release the glue-like substances, thereby mending the damaged region.

- Advantages:
  - Able to save water, able to save the environment from being damaged.
  - Able to mend the damaged pipe at early stage; if left not mended for longer, far greater damage might result.
  - Able to save water, able to save the environment from being damaged.
  - Able to mend the damaged pipe at early stage; if left not mended for longer, far greater damage might result.
- Disadvantages:
  - Having bacterial cells purposefully put into water for mending water pipes would be having bacteria in drinking water (might have some health issues).
  - Although the bacterial cells are to be dead, there is a possibility that there are some bacterial cells survived. There is a possibility of mutation that can lead to diseases. -Although this solution is dependent on production of glue-like substances upon quorum sensing, there might still be aggregation of bacterial cells and their by-products before reaching the damaged site. This may lead to clogging up the pipe.
- Limitations:
  - Leak in the pipe is very small (microscopic). Targeting the microscopic leak in the pipe would be difficult for the bacterial cells to carry out.
  - The water pipe or industrial pipe being used can be very large and long. Using the bacterial cells to target the leak and move to the damaged site via chemotaxis would be difficult to be carried out.

# Scenario H (Effective delivery of drugs to different parts of the body)

There are challenges faced with delivering drugs to the guts. There are possibilities that medicines taken orally are digested in the stomach before ever reaching the intestines. There is a need for drugs that are less prone to digestion whilst delivering drug effectively to the intestines.

- Applying the concept of synthetic biology, what possible solutions are there in order to tackle this problem?
- Also, when constructing the possible solution, think about its
  - advantages/disadvantages
  - limitations
  - feasibility.
- What other backup solutions do you have?

## Past Team Solution

Investigated by Imperial College 2009 iGEM Team.

They made use of bacterial cells to deliver drugs (or molecules that can treat the diseases) to the intestines.

1. In order to tackle the main problem, that is, not to get digested in the stomach, they made the bacteria to produce a protective layer around its cell body such that it prevents the digestion in the stomach by acid.
2. In order to prevent detrimental effects that could be caused by bacterial cells within the body (bacterial infection, body immune response to bacteria), bacterial genome deletion was carried out by cutting the genome into fragments by restriction enzyme (Would high school students be aware of the concept of restriction enzyme?). In this way, bacterial cells were not viable.
3. Drugs that were to be delivered to the intestines were made to be synthesized within bacterial cells (applying synthetic biology here). In the end, they had bacterial cells full of drugs of interest, coated in protective layer against acid, and non-viable. (Remember: protective layer is only specific for acid environment. It would not be effective in intestine, which has basic environment)

- Advantages:
  - Effective delivery can be done because the bacterial cells are protected from acid in the stomach.
  - Since the synthesis of drugs and protective layer (against acid) are both carried out in bacterial cell itself, it is more cost-effective. There would be no requirement for expensive sequential purification process for drugs.
- Disadvantages:
  - Although the bacterial cells are non-viable, there is a possibility that they can still be recognized by body immune system as foreign and get attacked.
  - There may be bacterial cells that retain their genome. This would not only make them viable but also, they can divide and produce lots more bacterial cells carrying drugs. This would result in over-dosage of drugs.