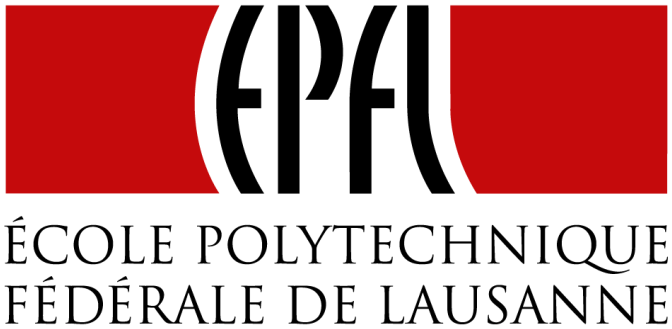
Biodesign for the real world

Falco ENZLER and Bastien ORSET



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General overview

- What is Biodesign?
- What is our kit and why make a new one?
- What about the legal aspects?
- An example of approved field kit: ARSOlux
- What about confinement?
- DNA construct details
- Future Design

Biodesign Project

- Biodesign is a multidisciplinary project with different research aspects :
  - development of field compatible water quality sensing systems
  - portable real-time sensing
  - crowd-sourced mapping

This project is a collaboration between people from India, Indonesia and Switzerland

- We are students in our third year of life science bachelor at the EPFL and we joined the Biodesign's team for our bachelor's project.

http://biodesign.cc/

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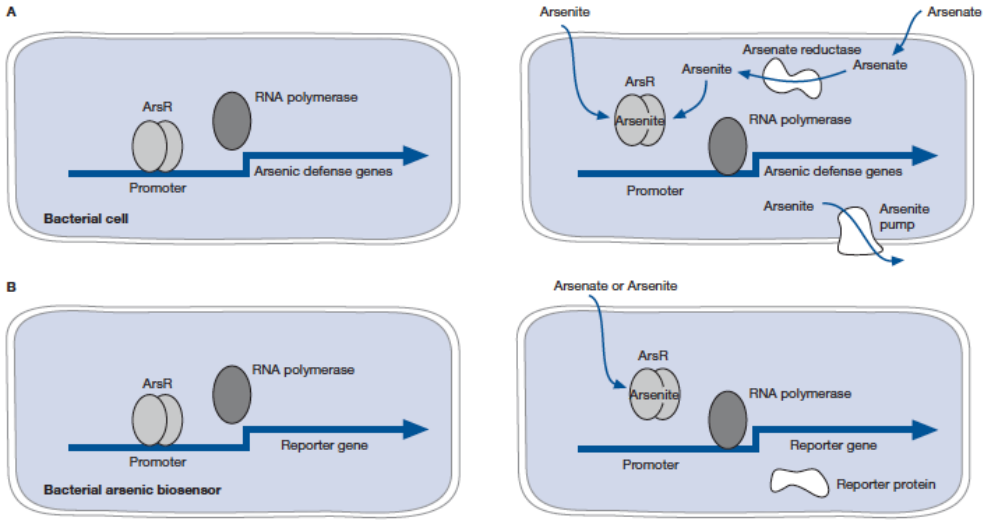
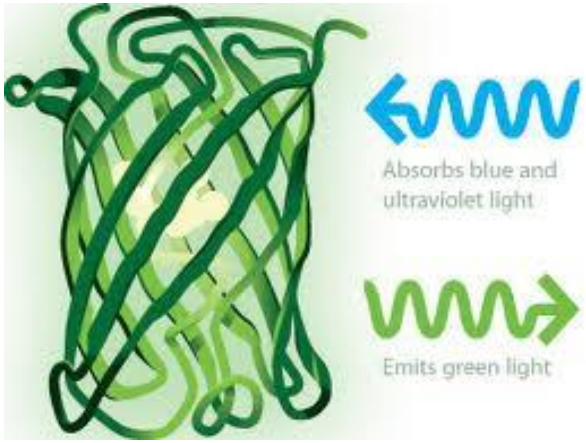
What is our project?

- The aim of this project is to detect arsenic in water samples in the field.
- This detection is accomplished with GMO bacteria expressing GFP (Green Fluorescent Protein) in presence of arsenic.
- Our prototype will allow to quantify GFP i.e. arsenic concentration





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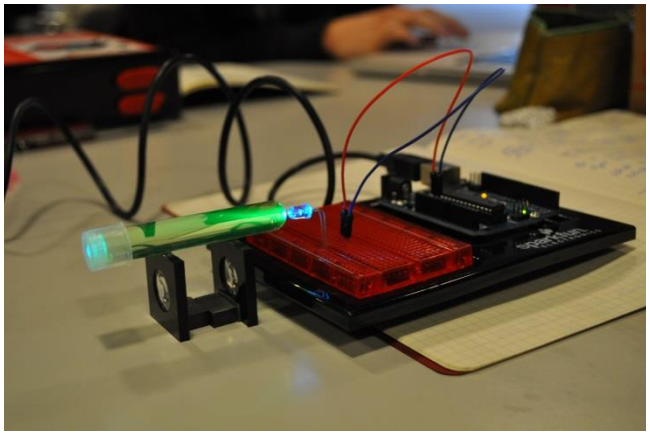
Why develop a new field kit?

Pros & Cons

What is our kit?

open hardware

- Community based / Understanding of the prototype
- Reparable



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What about legal aspects?

- The test kit would use genetically modified bacteria outside a qualified laboratory
- The main issue is to have the GMO confined from the environment.
- We thus refer to the « Ordinance on Handling Organisms in Contained Systems» 814.912
- According to Art.7, our kit is of class 1 i.e. null or negligible risk activity.

An example of approved GMO field kit: ARSOlux

- Detection of arsenic via bioluminesence
- Use of a freeze-dried GMO bioreporter
- Same vial
- Approved by the German authorities
- Tested in Bangladesh and other countries

http://www.ufz.de/arsolux/index.php?en=20706

http://www.ufz.de/export/data/411/33860_111005_ARSOlux_Projektkennblatt_englisch.pdf

Sigfried et al. Environ Sci Technol 2012 46(6) pp.3281-87

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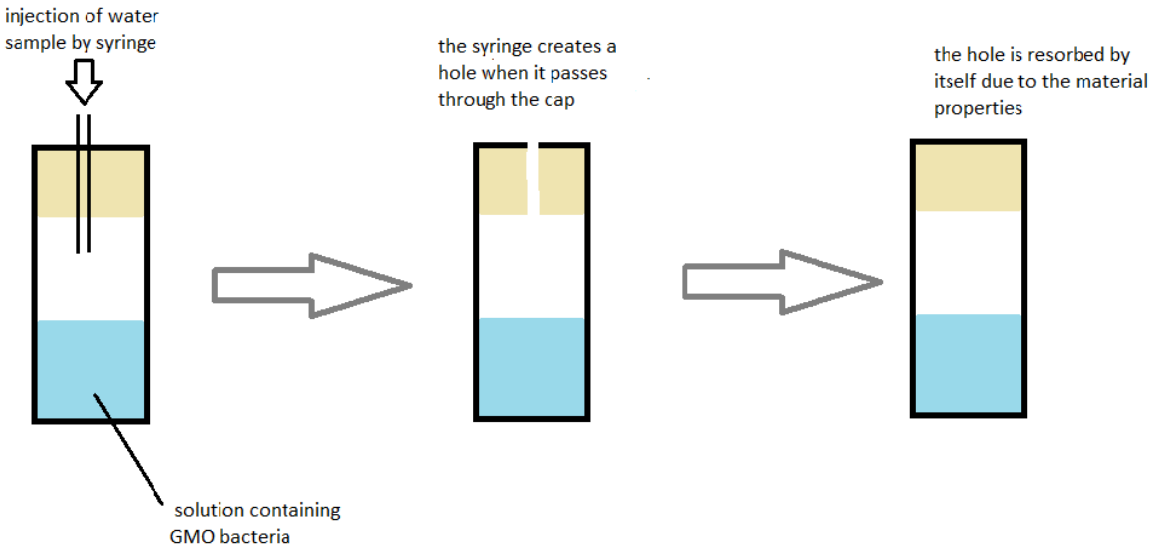
What about confinement?

- *"**contained system** means a system that uses physical barriers or a combination of physical and chemical or biological barriers to limit or prevent contact between organisms and people or the environment "*(Art.3,h.)



- After use, Bacteria and antibiotic are destroyed in the lab by autoclaving or inactivated by hypochlorite solution.

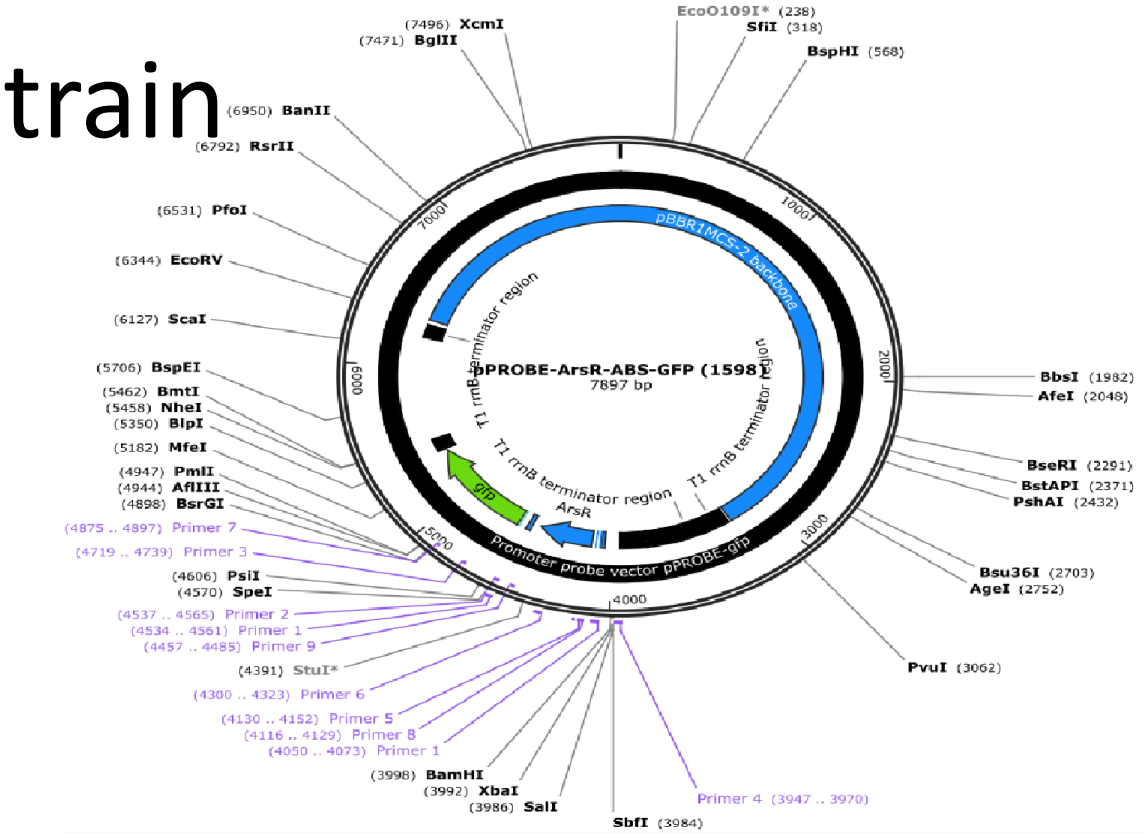
Details of the GMO arsenic reporter strain

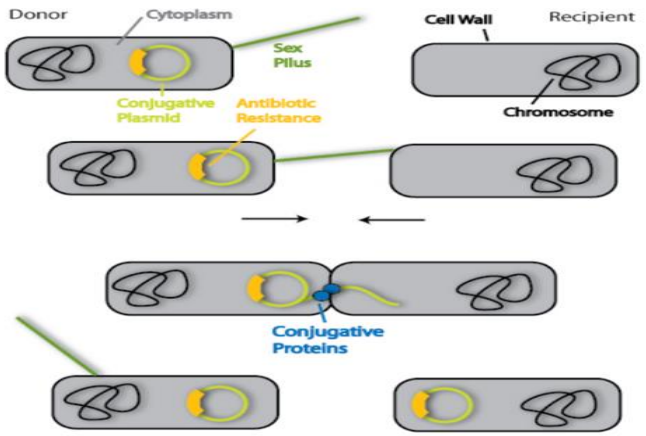
K-12 E.Coli bacteria strain (similar to ARSOlux:
E. coli DH5α)

- Can't do conjugation due to missing F factor and deficient recA (biological barrier)

Plasmid Design

- the plasmid carries kanamycin resistance, for easy selection





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Risk Analysis

- What happens if these GMO bacteria are released in the field? Could Kanamycin resistance and the arsenic reporting system be spread?

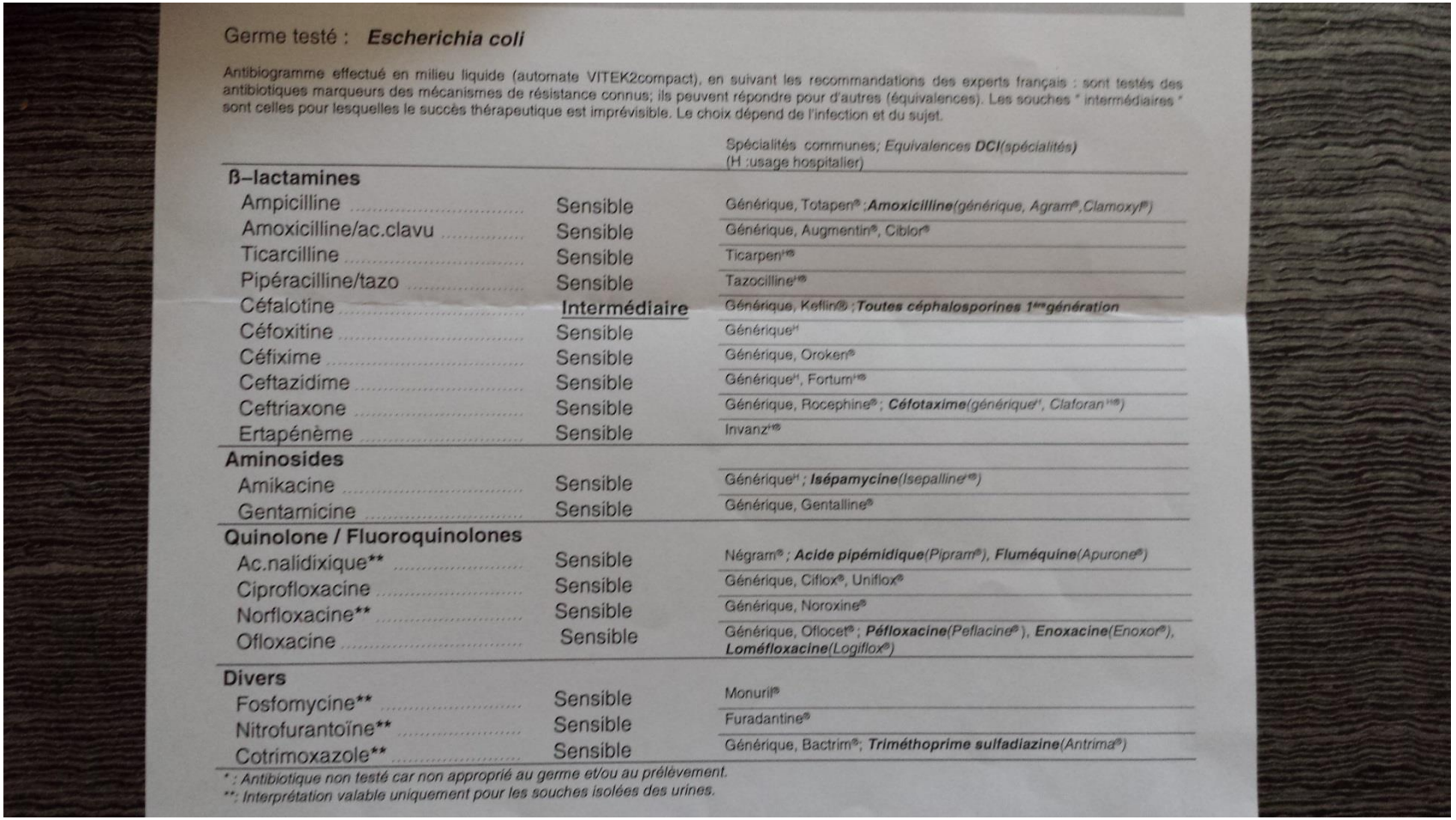
-> This E. coli strain cannot transfer the plasmid by itself to other bacteria.

Should cells lyse, the plasmid DNA can be liberated and theoretically be taken up by naturally transformable strains.

- GFP non toxic and its fluorescence is not dangerous for the cell.
- Other drugs exist to kill Kanamycin resistant bacteria.

-> This resistance could be removed but the detection efficiency would decrease.

Antibiogram



Germe testé : ***Escherichia coli***

Antibiogramme effectué en milieu liquide (automate VITEK2compact), en suivant les recommandations des experts français : sont testés des antibiotiques marqueurs des mécanismes de résistance connus; ils peuvent répondre pour d'autres (équivalences). Les souches " intermédiaires " sont celles pour lesquelles le succès thérapeutique est imprévisible. Le choix dépend de l'infection et du sujet.

		Spécialités communes; *Equivalences* ***DCI****(spécialités)* (H :usage hospitalier)
ß–lactamines		
Ampicilline	Sensible	Générique, Totapen® ;***Amoxicilline****(générique, Agram®,Clamoxyl®)*
Amoxicilline/ac.clavu	Sensible	Générique, Augmentin®, Ciblor®
Ticarcilline	Sensible	Ticarpen[H®]
Pipéracilline/tazo	Sensible	Tazocilline[H®]
Céfalotine	Intermédiaire	Générique, Keflin® ;***Toutes céphalosporines 1ère génération***
Céfoxitine	Sensible	Générique[H]
Céfixime	Sensible	Générique, Oroken®
Ceftazidime	Sensible	Générique[H], Fortum[H®]
Ceftriaxone	Sensible	Générique, Rocephine® ; ***Céfotaxime****(générique[H], Claforan[H®])*
Ertapénème	Sensible	Invanz[H®]
Aminosides		
Amikacine	Sensible	Générique[H] ; ***Isépamycine****(Isepalline[H®])*
Gentamicine	Sensible	Générique, Gentalline®
Quinolone / Fluoroquinolones		
Ac.nalidixique**	Sensible	Négram® ; ***Acide pipémidique****(Pipram®)*, ***Fluméquine****(Apurone®)*
Ciprofloxacine	Sensible	Générique, Ciflox®, Uniflox®
Norfloxacine**	Sensible	Générique, Noroxine®
Ofloxacine	Sensible	Générique, Oflocet® ; ***Péfloxacine****(Peflacine®)*, ***Enoxacine****(Enoxor®)*, ***Loméfloxacine****(Logiflox®)*
Divers		
Fosfomycine**	Sensible	Monuril®
Nitrofurantoïne**	Sensible	Furadantine®
Cotrimoxazole**	Sensible	Générique, Bactrim®; ***Triméthoprime sulfadiazine****(Antrima®)*

** : Antibiotique non testé car non approprié au germe et/ou au prélèvement.*
***: Interprétation valable uniquement pour les souches isolées des urines.*

Future Design & Possibilities

- Turbidity measurement to normalize the GFP signal
- Electronic system incorporated inside the box
- Built-in interface (buttons, screen…)
- Use with a smartphone via an application
- Adaptable to other bioreporters for harmful elements detection (Mercury etc…)
- Field test

Answer to possible questions

Why As is not good? Where?

- Interfere with Krebs cycle (inhibits pyruvate conversion to acetyl-coA)
- As a slow poison, causes diseases
  - Skin diseases
  - Intestinal tract problems
  - Cancers
- Maximum concentration advised by WHO: 10µg/L
  - Letal dose: 1mg/kg/day
- Problem in Bangladesh and some Asian countries

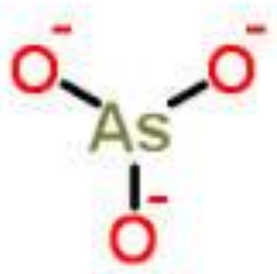
What is our current cost?

- Around 50 CHF
- Possibility to develop a cheaper prototype using another micro-controller, i.e a simpler Arduino hardware.

Why not detect bioluminescence?

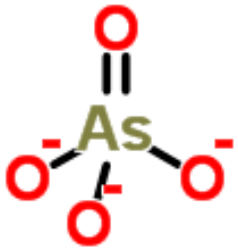
Difference between arsenite and arsenate?

- Arsenite has less oxygen than Arsenate



Arsenite
AsIII
(As_2O_3, AsO_2^-, AsO_3^{3-})

Reporter more sensitive



Arsenate
AsV
(AsO_4^{3-})