Biodesign for the real world

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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/
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
Why develop a new field kit?

Pros & Cons
What is our kit?

- Community based / Understanding of the prototype
- Reparable
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 bioluminescence
- Use of a freeze-dried GMO bioreporter
- Same vial
- Approved by the German authorities
- Tested in Bangladesh and other countries


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: \textit{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
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

<table>
<thead>
<tr>
<th>Germe testé : Escherichia coli</th>
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<tbody>
<tr>
<td>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.</td>
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<tr>
<th>B--lactamines</th>
<th>Sensible</th>
<th>Spécialités communes ; Equivalences DCI(specialités)</th>
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<tbody>
<tr>
<td>Amoxicilline</td>
<td></td>
<td>Générique, Totapen® : Amoxicilline(générique, Agram®, Clamoxy®)</td>
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<tr>
<td>Amoxicilline/ac.clavu</td>
<td></td>
<td>Générique, Augmentin®, Cibior®</td>
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<td>Ticarcilline</td>
<td></td>
<td>Ticarpen®</td>
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<td>Pipéracilline/tazo</td>
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<td>Tazocilline®</td>
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<tr>
<td>Céfaiotine</td>
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<td>Générique, Keflin® ; Toutes céphalosporines 1ère génération</td>
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<tr>
<td>Céfoxitine</td>
<td></td>
<td>Générique®</td>
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<td>Céfixime</td>
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<td>Générique, Orceen®</td>
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<tr>
<td>Céfazidime</td>
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<td>Générique®, Fortum®</td>
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<td>Ceftriaxone</td>
<td></td>
<td>Générique, Rocephine® ; Céfotaxime (générique®, Claforan®)</td>
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<td>Ertapénème</td>
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<td>Invanz®</td>
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<th>Aminosides</th>
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<tr>
<td>Amikacine</td>
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<td>Générique® ; Isépamycin (Isepalline®)</td>
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<td>Gentamicine</td>
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<td>Générique, Gentalline®</td>
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<tr>
<th>Quinolone / Fluoroquinolones</th>
<th>Sensible</th>
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<tbody>
<tr>
<td>Ac.nalixique**</td>
<td></td>
<td>Néogram® ; Acide pipéridique (Pipram®), Fluméquine(Apurone®)</td>
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<tr>
<td>Ciprofloxacine</td>
<td></td>
<td>Générique, Ciflox®, Uniflox®</td>
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<td>Norfloxacine**</td>
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<td>Générique, Noroxine®</td>
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<td>Ofloxacine</td>
<td></td>
<td>Générique, Olcocet® ; Pefloxacine® (Peflacin®), Enoxacine (Enoxor®), Loméfloxacine (Logiflox®)</td>
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<th>Divers</th>
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<td>Fosfomycine**</td>
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<td>Monuril®</td>
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<td>Nitrofurantoine**</td>
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<td>Furadantine®</td>
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<td>Cotrimoxazole**</td>
<td></td>
<td>Générique, Bactrim® ; Triméthoprime sulldiazine(Antrimat®)</td>
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* : 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
ArSIII
(As$_2$O$_3$, AsO$_2^-$, AsO$_3^{3-}$)

REPORTER more sensitive

ARSENATE
AsSV
(AsO$_4^{3-}$)