

# A whole cell bioluminescent biosensor on a chip for on line detection of cadmium and other heavy metals in freshwater

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## Introduction :

**Problematic :** Cadmium, a heavy toxic metal known as carcinogen, is among the 33 pollutants recognized as priority by the European Community in the field of water policy (1). In drinking water, the authorized concentration of cadmium is  $5\mu\text{g}\cdot\text{L}^{-1}$  (45nM).

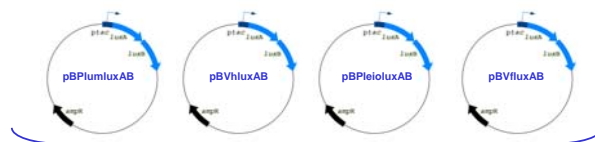
**Purpose :** In order to monitor cadmium as well as other toxic metals, we developed a recombinant bioluminescent bacteria immobilized on a disposable chip. The latter was introduced into a portable device namely Lumisens2, a new versatile biosensor able to use every bioluminescent strains. Bacterial biosensors are a simple alternative to the chemical methods for water analysis, like *E.coli*/TBT3, lately used for TBT detection (2, 3).

### Processes :

- In order to chose a high and stable bioluminescent genetic reporter system, we have studied the light production of four different luciferases genes (*luxAB* from *Vibrio harveyi*, *V.fischeri*, *Photobacterium luminescens* and *Photobacterium leiognathi*) cloned in the same structure and transformed in *E.coli* XL1.
- The heavy metal inducible promoter *pzntA* (4) was cloned upstream the selected *lux* operon. The plasmid obtained was then introduced in *E.coli* and the detection of cadmium was optimised.
- The strain was immobilized in Lumisens2 for the online control of cadmium concentration.

## Step 1 : Choice of a bioluminescence operon

> Cloning of the four different *luxAB* luciferase genes upstream the *ptac* constitutive promoter (without *lacI* in a multicopy expression) in pBtac2



Study of the bioluminescence of each construction in the same strain *E.coli* XL1 with :

- > The addition of three different aldehydes : nonanal, decanal and dodecanal
- > Two different temperatures : 30 and 37°C

### Results

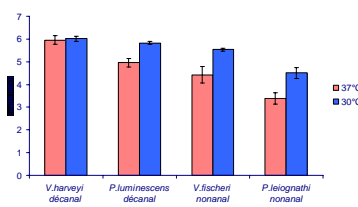


Figure 1: Conditions for the maximum bioluminescence emission for each construction in *E.coli* XL1

If we considered the level of light production, the classification is: *V.harveyi* > *P.luminescens* > *V.fischeri* > *P.leiognathi*. As a consequence the *lux* operon from *V.harveyi* should be chosen. Due to the complexity of its restriction map, this operon is not easy to use. Therefore the *lux* operons from *V.fischeri* and *P.luminescens* were selected.

## Step 2 : Cloning *pzntA* and optimisation of the detection

> Cloning the inducible promoter *pzntA* and effect of the host strain : *E.coli* XL1 or DH1.

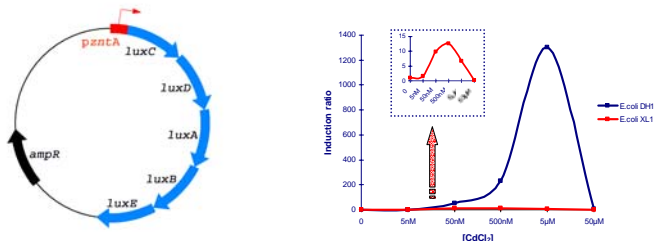


Figure 2: Map of the plasmid pBzntlux designed for the detection of heavy metals with *E.coli* type strains.

Figure 3: Light production of strains *E.coli* DH1 and XL1 pBzntlux for several concentrations of  $\text{CdCl}_2$  in acetate medium. Standard errors are below 5% and hence are not shown.

> Specificity of *E.coli* DH1 pBzntlux

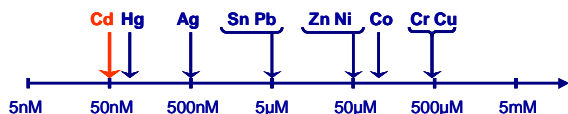


Figure 4: Specificity of *E.coli* DH1 pBzntlux for several metals

> Detection limit of *E.coli* DH1 pBzntlux in liquid phase and in agarose

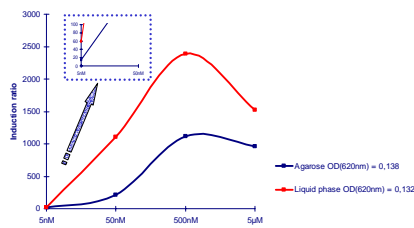


Figure 5: Induction ratio measured in acetate medium and in 2% agarose. Standard errors are below 5% and hence are not shown.

The plasmid pBzntlux, inserted in the selected strain *E.coli* DH1, detects a wide range of metals. The detection limit ( $5\text{nM}$  of  $\text{CdCl}_2$ ) is not affected by the immobilization of the strain in agarose and is under the environmental level.

## Conclusions and Perspectives :

- > Each step of the study allowed us to select the optimal conditions so that the sensible strain is able to detect cadmium on-line in the Lumisens2 biosensor.
- > The detection of cadmium is validated in the Lumisens2 biosensor.
- > In a future work, the detection of cadmium and other heavy metals will be optimised in the biosensor using different flow rates and cell concentrations in agarose as well as application to waste water effluent.

## Step 3 : Validation of the on line cadmium detection with Lumisens2

> Lumisens2 is a compact, portable and versatile biosensor using disposable chips into which cells are immobilized.

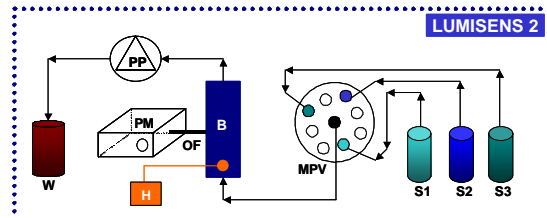


Figure 6: Schematic representation of LUMISENS 2. B biochip; H heater and temperature regulation; MPV multiposition valve; PM photomultiplier and amplifier; PP peristaltic pump; S1, S2, S3 sample or washing solutions; OF optical fibers; W waste

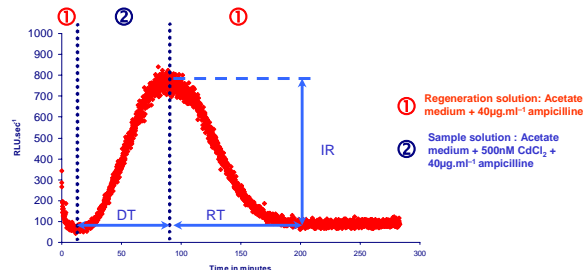


Figure 7: Response of the Lumisens2 biosensor with *E.coli* DH1 pBzntlux in agarose gel. Sequence used is : 15 min solution ①, 65 min solution ②, and return to solution ①. The experiment was performed at 30°C with a  $5\text{mL}\cdot\text{min}^{-1}$  flow rate. DT : Detection Time = 65min, RT Recovery Time = 110min, IR Induction Ratio = 10.

The measurement of cadmium in Lumisens2 is validated. The strain immobilized in 2% agarose gives a response characterised by a detection time of 65 minutes with a significant induction ratio.

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## References :

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