

BioBricks homework

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Background

Cadmium(Cd) is known to be used in insecticides, fungicides, and fertilizers and is also a byproduct of industrial processes such as smelting.

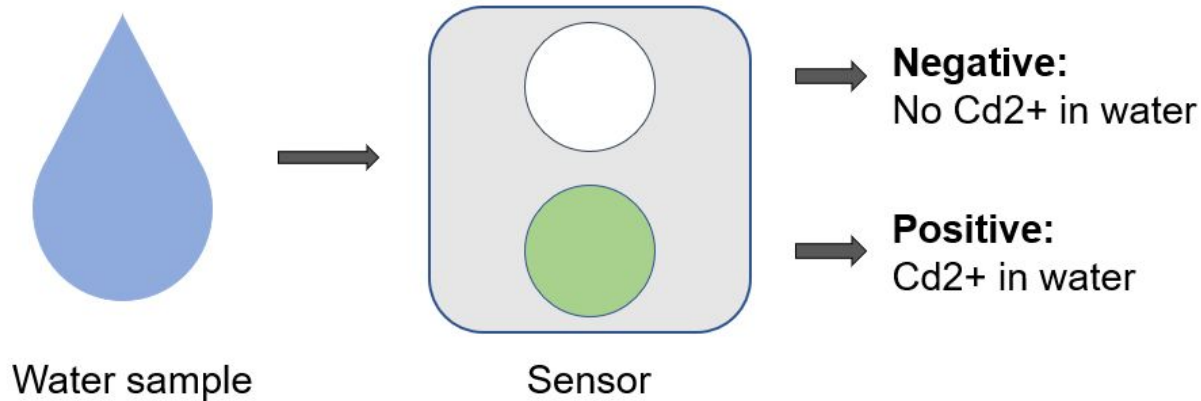
If ingested it can cause numerous health problems including cancer and pulmonary edema.

Despite improvements in water quality, contaminants still interfere with farming in many of the world's biomes.

Previous method to detect this metal is construction of the sensor by using a cadmium sensitive promoter and an activator which are upstream from a stronger promoter.

Sensor to detect cadmium

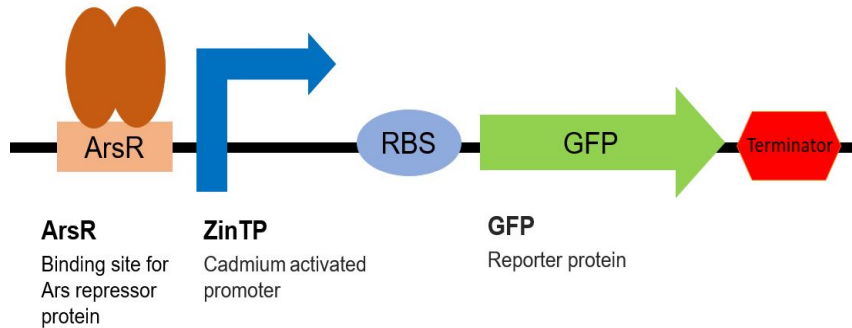
Whole cell sensor with *Escherichia Coli* cells



E. Coli cells will express green fluorescent protein (GFP) in the presence of Cd²⁺. Upon excitation, the sensor will either emit green light (positive samples) or not (negative samples).

Design

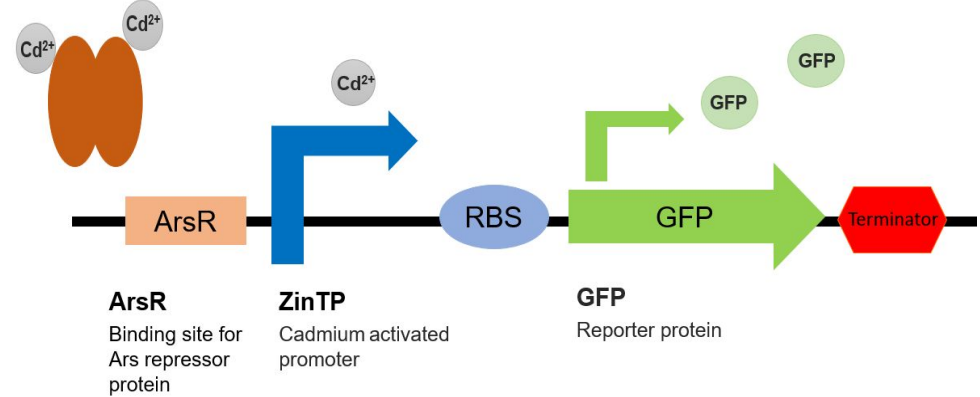
Off state



Repressor protein bound to DNA and promoter not activated

→ No GFP expression

On state



Cd²⁺ binds to repressor protein, and activates ZinT promoter

→ GFP expression

Together, the inducible promoter and ArsR repression will form a selective AND gate for the detection of cadmium

iGEM parts

ArsR binding site: BBa_K174016

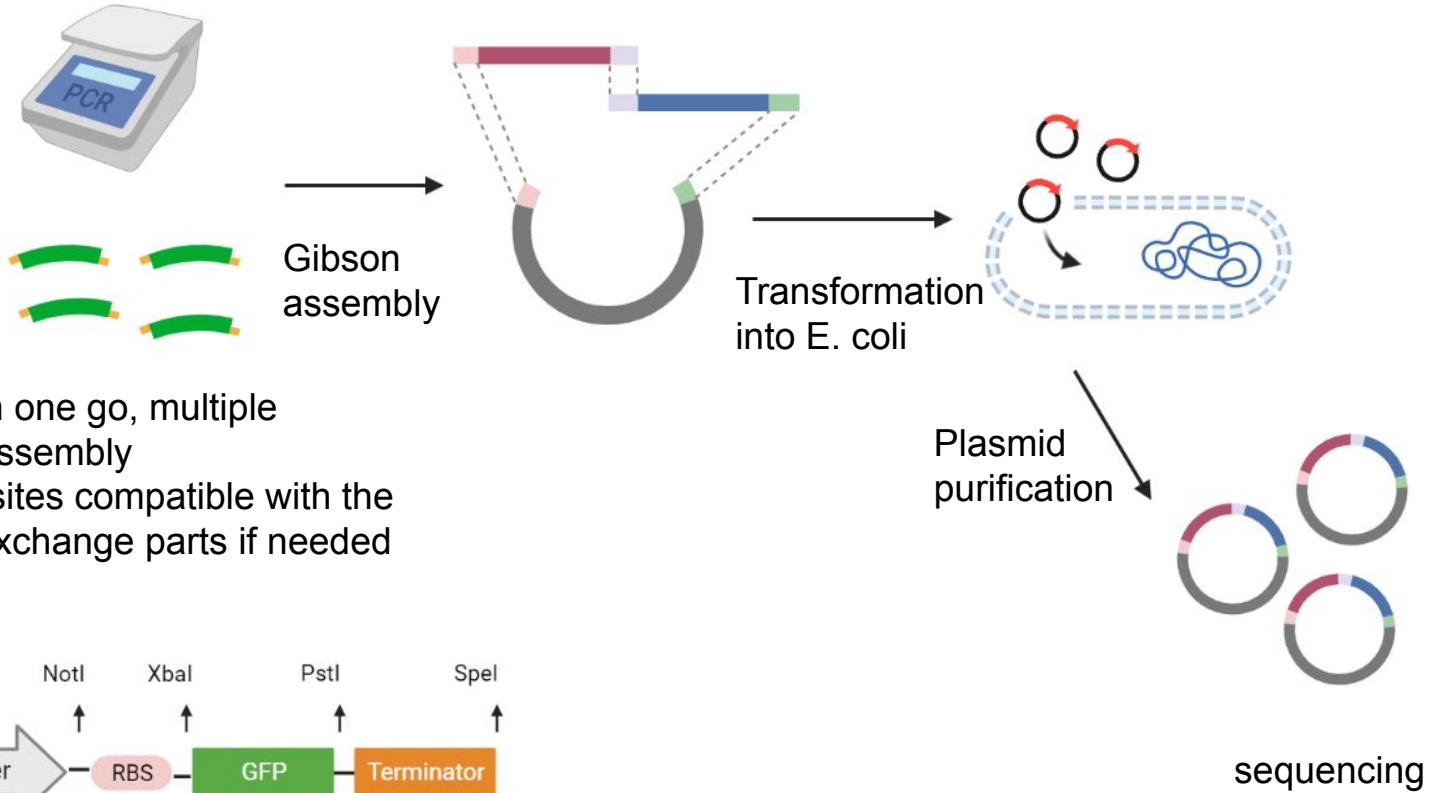
ZinT promoter: BBa_K896008

RBS: BBa_J61100

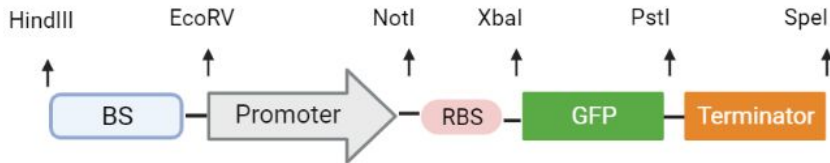
GFP: BBa_E0040

Terminator: BBa_B0015

Assembly



- Assembly easily in one go, multiple fragment gibson assembly
- Added restriction sites compatible with the RFC10 to easily exchange parts if needed with PCR primers



Truth table

- If there is cadmium in the wastewater, it will detach the repressor protein and activate the cadmium sensitive promoter transcribing GFP
- If there is no cadmium in the environment, there is no GFP signal

Cd ²⁺	GFP
0	0
1	1

Other considerations

For better sensitivity and resistance to heavy metals, additional parts can be added, e.g.,

- BBa_K231000 with constitute promoter: cadmium (and other metal) -binding peptide that increases the resistance against toxic metals and enhances the uptake of metal ions

For safety with GMOs, a kill switch could be designed so that the cells would not be able to survive outside the sensor.

References

A. Paskarova, P. Ferianc, J. Kormanec, D. Homerova, A. Farewell, T. Nystrom. Regulation of yodA encoding a novel cadmium-induced protein in Escherichia coli, *Microbiology* (2002), 148, 3801–3811

Hobman, J. (2015, July 7). Zinc dependence of Zint (YODA) mutants and binding of zinc, cadmium, and Mercury by Zint. *Biochemical and Biophysical Research Communications*. Retrieved October 10, 2022