Biosensor for mercury ions

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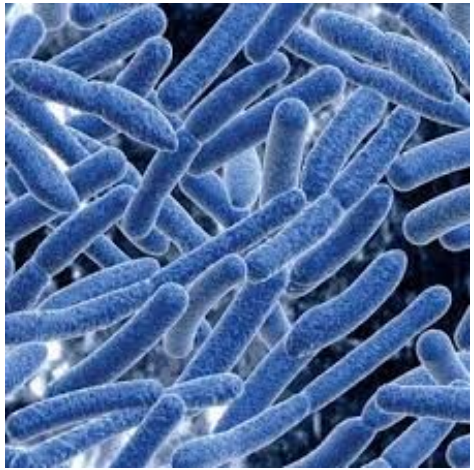
Introduction

Key facts about Mercury:

- One of the top 10 groups of chemicals of **major public health concern** (WHO)
- Element naturally found in air, water and soil
- Human activity is the major cause of **mercury contamination**
- Elemental (metallic), organic (methylmercury) and inorganic mercury (Hg^{2+})
- **Toxic effects** on the nervous, digestive and immune systems, on lungs, kidneys, skin and eyes
- **Developmental threat** to foetuses and children in early life
- Main exposure via methylmercury which bioaccumulates in fish and shellfish living in **contaminated water** and via inhalation during industrial processes
- Maximum contaminant level of inorganic mercury in water set at 2 µg/litre by the US Environmental Protection Agency for drinking-water

Mercury ions biosensor



- Design of a **biosensor** able to detect and quantify **mercury ions Hg^{2+}** in a sample
- Chassis used: E. coli

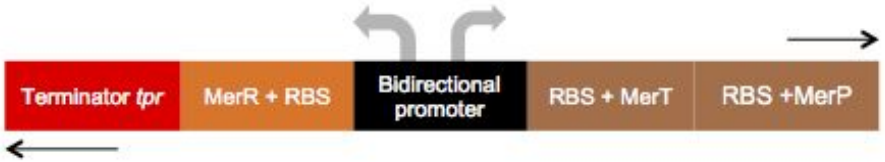
- Potential applications:
  - Assess contamination levels of a water source (lake, sea, river…)
  - Assess contamination of an organism via blood, urine or faeces sample

Parts

Selected parts (iGEM code numbers)

- **Regulation and transport of mercury ions (BBa_K1355001)**
- **Blue chromoprotein, aeBlue (BBa_1033929)**
  - includes RBS
- **Backbone (pSB4K5)**

Regulation and transport of mercury ions



- BBa_K1355001
- key piece to detect Hg^{2+}
- bidirectional promoter
- MerR controls expression of MerT and MerP
- Hg^{2+} binds to MerR, allowing MerPT expression
- MerP: carrier protein, Hg^{2+} from periplasm to inner membrane
- MerT: carrier protein, Hg^{2+} from inner membrane to cytoplasm
- iGEM UFAM Brazil 2014

http://parts.igem.org/Part:BBa_K1355001

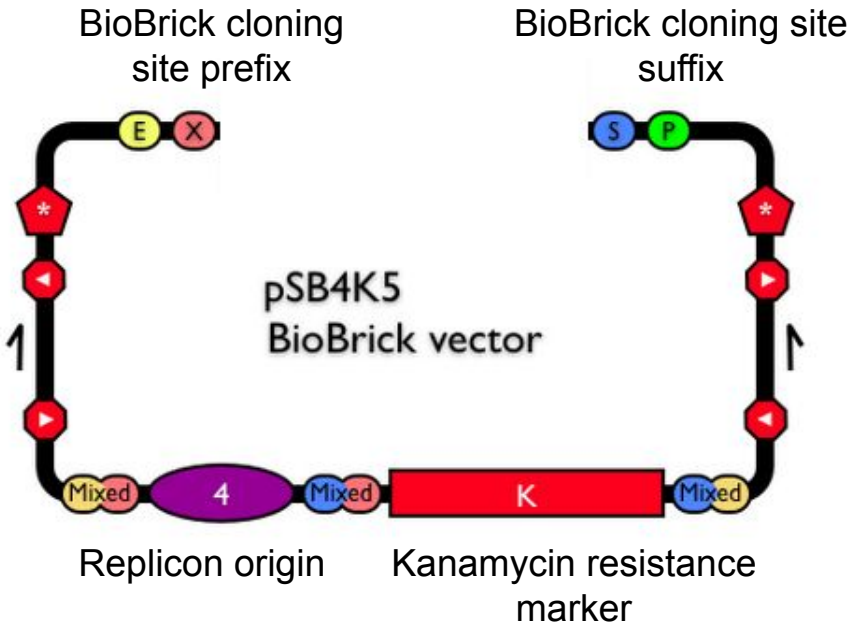
Blue chromoprotein

- BBa_K1033929
- iGEM Uppsala 2013
- From beadlet anemone *Actinia equina*
- Codon optimized for *E. coli*
- Visible after ~24 hours
- Absorption maximum at 597 nm
- Good visual detection, clear colour, easy to see
- colourblind friendly
- includes RBS

http://parts.igem.org/Part:BBa_K1033929

Backbone

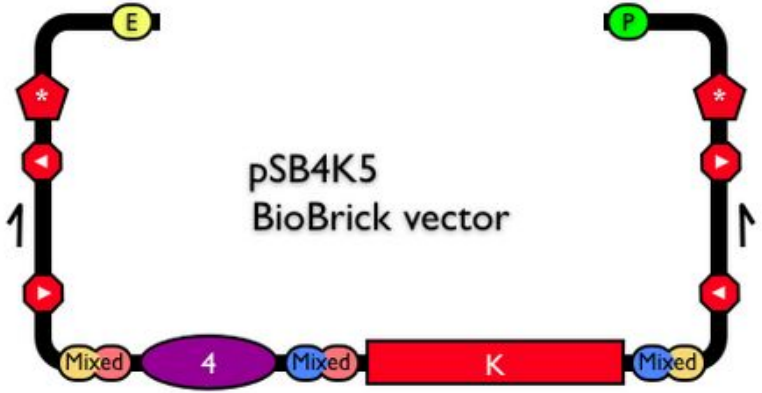
- Backbone: pSB4K5
  - Replicon: pSC101 (BBa_I50042)
  - Selection marker: Kanamycin resistance (BBa_P1003)
- Copy number: ~5
- We chose this backbone because its copy number is low. This decreases the stress on the cells.



Assembling

- BioBrick RFC[10] standard
- Can be done simply in one round.

Symbol	Restriction Enzyme
E	EcoRI
X	XbaI
S	SpeI
P	PstI



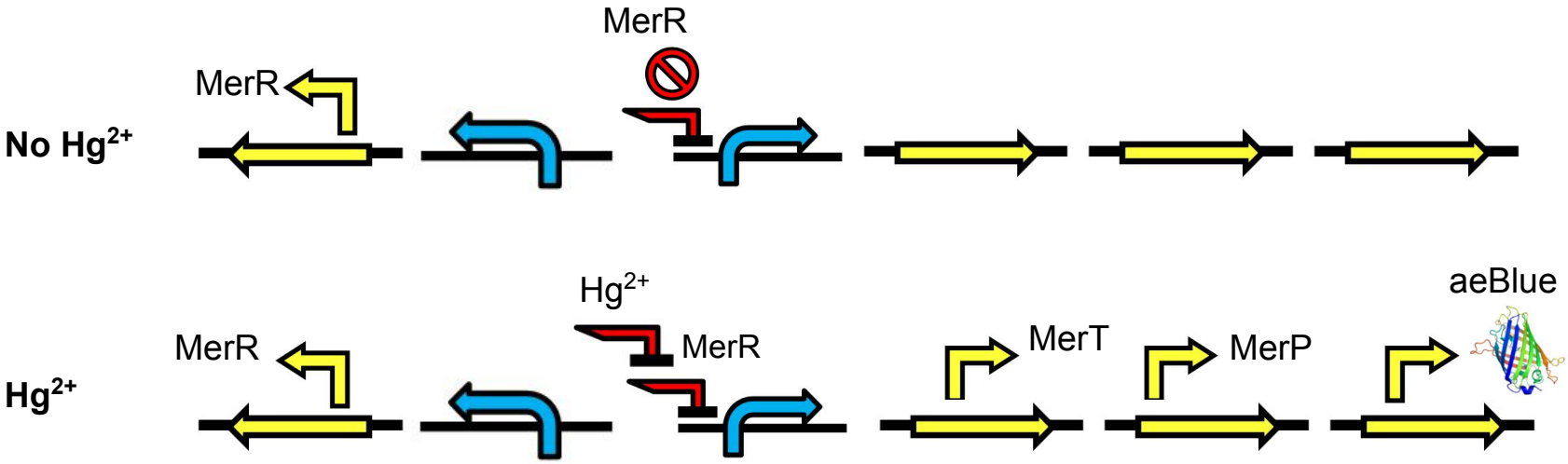
How the system works?

OFF: Hg^{2+} absent, MerR forms a promoter-operator complex that prevents RNA polymerase from recognizing the promoter and hence the MerTP proteins and the chromoprotein (aeBlue) will not be transcribed.

ON: Hg^{2+} present, MerR binds to Hg^{2+} and dissociates from the promoter-complex allowing the transcription and expression of the MerTP and the blue chromoprotein (aeBlue).

Hg^{2+}	MerTP	aeBlue
0	0	0
1	1	1

Pathway schematic



Conclusion

What are the main advantages of our new biosensor ?

UV light is not needed to observe the color change

→ Samples don’t have to be analyzed in a lab

→ Application more affordable for developing countries

References

https://www.who.int/en/news-room/fact-sheets/detail/mercury-and-health

Mercury in Drinking-water - Background document for development of WHO Guidelines for Drinking-water Quality

http://parts.igem.org/Part:BBa_K1355001

http://parts.igem.org/Part:BBa_K1033929

http://parts.igem.org/Part:pSB4K5

http://parts.igem.org/Help:Standards/Assembly/RFC10