

Standardization, biobricks and chassis CHEM-E8125



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WHAT IS A BIOSENSOR?

- An analytical device, used for the detection of a chemical substance
- The bioreceptor interacts with a specific analyte of interest to produce an effect measurable by the transducer
- Can detect very low concentrations of substances such as drugs, toxins or explosives

WHAT IS A BIOBRICK?

- A DNA part in a standard format with a function that can be quantified
- Building blocks for biosensors
- The Registry of Standard Biological Parts

WHAT KIND OF SENSOR WE WANT?

- A metal detecting biosensor (*E. coli*) that detects heavy metals
- Promoters inducible by metal ions
- Expresses fluorescent proteins RFP, GFP and blue chromoprotein in the presence of these metal ions

Heavy metals

- Our biosensor detects lead, cadmium and mercury and produces chromoproteins in response.
- **Why are heavy metals bad?**

- Lead is toxic mainly because it preferentially replaces other metals (e.g., zinc, calcium and iron) in biochemical reactions in living organisms
e.g. it displaces calcium in the reactions that transmit electrical impulses in the brain. This can have fatal consequences for the organism.
- Mercury accumulates to kidneys and brains in the human body and affects especially to the nervous system. Exposure to mercury can happen by inhaling mercury contaminated air, orally or it can even absorb through the skin.
- Mercury can affect brain function in various ways (like sensory impairment), it causes acrodynia, Hunter-Russell syndrome, Minamata disease and damages the kidneys
- Cadmium binds to red blood cells in body and accumulates to kidneys and enlarges individual risk for kidney diseases. Cadmium is also a known carcinogen.
- It is suspected that cadmium replaces zinc in some enzymes due to their chemical similarity.

Biosensor design

Under constitutive promoter only single mRNA produced, proteins separated by self-cleaving T2A peptides (BBa_K1993019).

Double terminator (BBa_B0015) used to ensure ending of the transcription.

RFC[10] compatible plasmid construction.

Heavy metal detection via **chromoproteins**



Plasmid construct

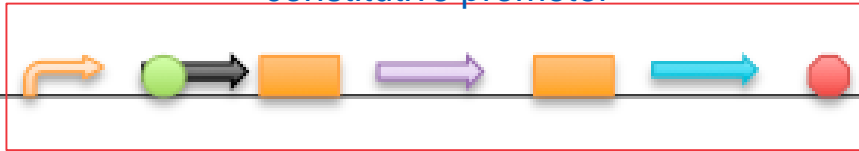
- **pSB1C3** plasmid backbone
- Chloramphenicol resistance
- The replication origin is a pUC19-derived pMB1
- High copy number (100-300 per cell)

Parts used in plasmid assembly

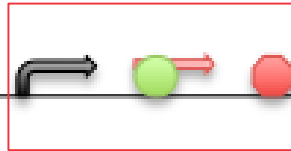
Name	Description	Length
pSB1C3	plasmid backbone with prefix, suffix, chloramphenicol resistance gene and pUC19-derived pMB1 replication of origin pSB1C3	2070
BBa_J23100	constitutive promoter family member	35
BBa_I721001	lead + protein inducible promoter	94
BBa_M36245	PCad Promoter	
BBa_K346002	PmerT promoter (mercury-responsive)	57
BBa_I721002	Lead Binding Protein	399
BBa_M36244	CadC Transcription Factor	
BBa_K1420004	merR family transcriptional regulator protein	435
BBa_E1010	engineered mutant of red fluorescent protein	706
BBa_K1789003	GFP1	474
BBa_K864401	aeBlue blue chromoprotein	699
BBa_B0034	Ribosomal Binding Site	
BBa_K1993019	T2A cleavage site	18–22
BBa_B0015	Double Terminator	80

Gene circuit construction

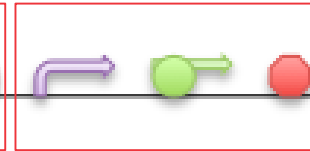
Inducer proteins produced under
constitutive promoter



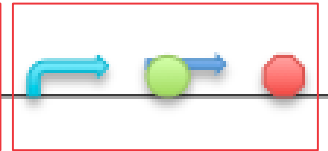
Lead detection



Cadmium detection



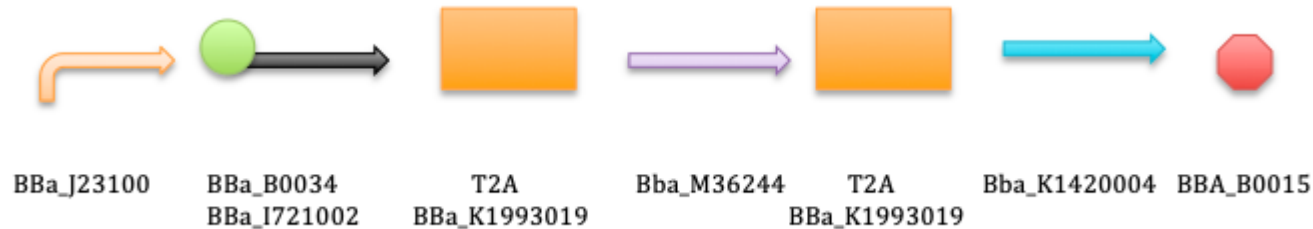
Mercury detection



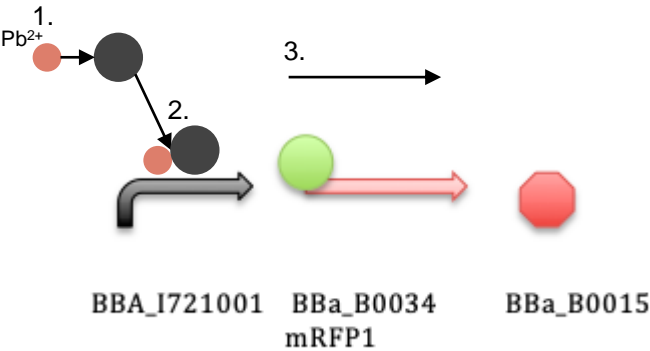
Constitutive

Under a single constitutive promoter, three different proteins (lead binding protein, CadC, merR) are produced.

T2A peptides cleave the proteins apart from each other after translation of mRNA.



Lead detection

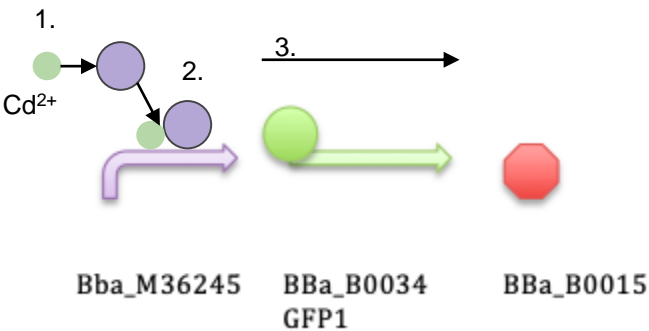


1) Lead ion (Pb^{2+}) and lead binding protein produced under the constitutive promoter form a dimer.

1) Dimer binds to the lead inducible promoter and derepresses it.

1) Translation of the RFP protein sequence is initiated.

Cadmium detection

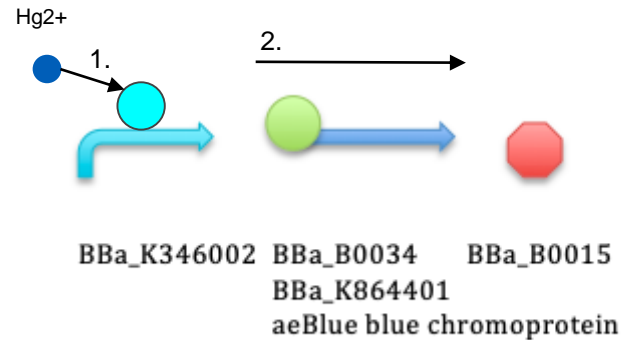


1. Cadmium ion (Cd^{2+}) and cadmium transcription factor (CadC) produced under the constitutive promoter form a dimer.

1. Dimer binds to the CadC inducible promoter and derepresses it.

1. Translation of the GFP protein sequence is initiated.

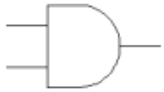
Mercury detection



1. Under the constitutive promoter produced merR forms a homodimer and binds to the mercury inducible promoter. When mercury ion (Hg^{2+}) is present, it binds to the homodimer.

1. Metallated MerR homodimer causes a realignment of the promoter after which translation of the blue chromoprotein sequence is initiated.

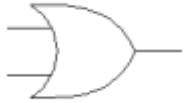
Logic Gates



Const. promoter	RFP	GFP	aeBlue
0	0	0	0
1	1	1	1

AND: The output of an AND gate is true when all its inputs are true ie. all ions are present.

Logic Gates



Const. promoter Ion	Pb ²⁺	Cd ²⁺	Hb ²⁺	Fluorescent protein produced
0	0	0	0	0
1	1	0	0	RFP
1	1	1	0	RFP, GFP
1	1	0	1	RFP, aeBlue

OR: The output of an OR gate is true when at least one of its inputs is true ie. one or more ion is present.

References

- Amaro F, Turkewitz AP, Martín-González A, Gutiérrez JC. Whole-cell biosensors for detection of heavy metal ions in environmental samples based on metallothionein promoters from *Tetrahymena thermophila*. *Microb Biotechnol*. 2011;4(4):513-22.
- Verma, Neelam & Singh, Minni. (2005). Biosensors for heavy metals. *Biometals : an international journal on the role of metal ions in biology, biochemistry, and medicine*. 18. 121-9. 10.1007/s10534-004-5787-3.
- <http://parts.igem.org>

Thank You!