BioBricks: Lead and mercury sensor

BIOBRICKS GROUP 4

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Background and motivations

- Lead and mercury are neurotoxins
- ➢ Water, as lead source, is one the largest controllable sources (WHO)
- Due to pollution both can be found in nature
- Sensory system that can detect both metals individually or as a pair
- Our system is affordable bio-based solution

Selected parts

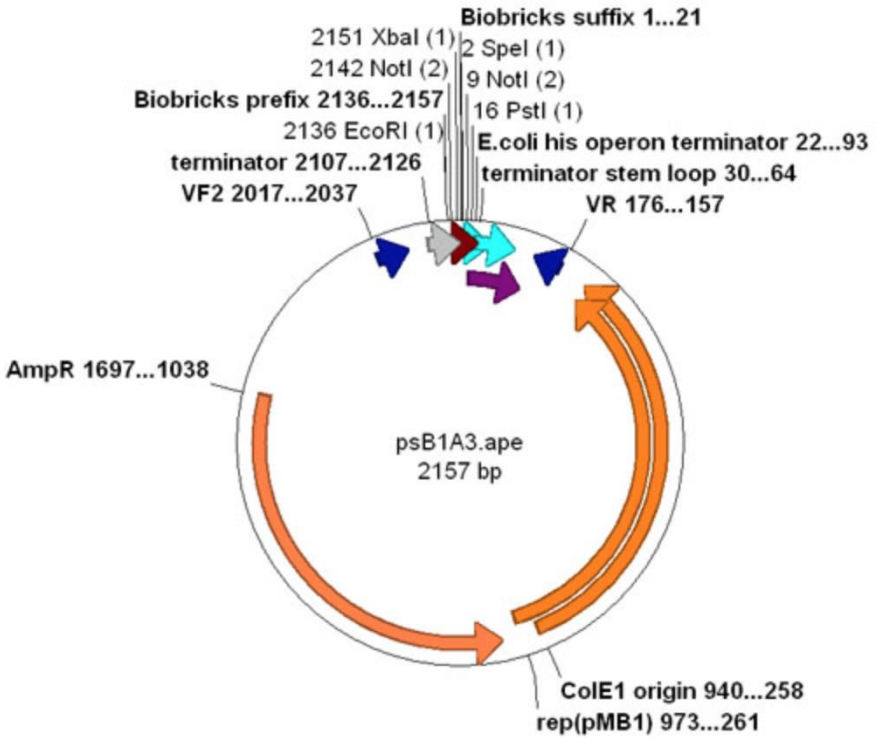
Constitutive promoters: Promoter J23100 (BBA_J23100), promoter J23119 (BBa_J23119)

Promoters: Lead promoter (BBa_I721004), Mercury promoter **PmerT** (BBa_K346002)

Plasmid backbone pSBIA3 with **AmpR**

Assisting genes: Lead binding protein (BBa_I721002), Mercury-responsive transcription factor **MerR** (BBa_K346001)

Reporters: GFP (BBa_E0040), RFP (BBa_E1010)

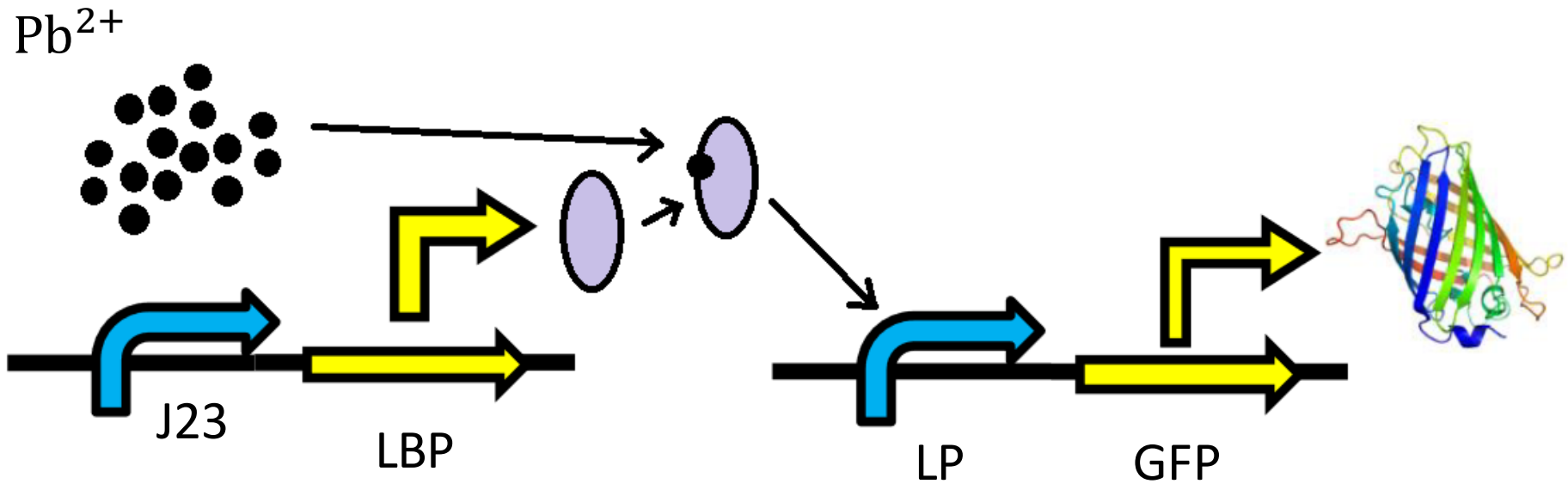


http://parts.igem.org/Part:pSB1A3

Lead promoter (BBa_I721004) and lead binding protein (BBa_I721002)

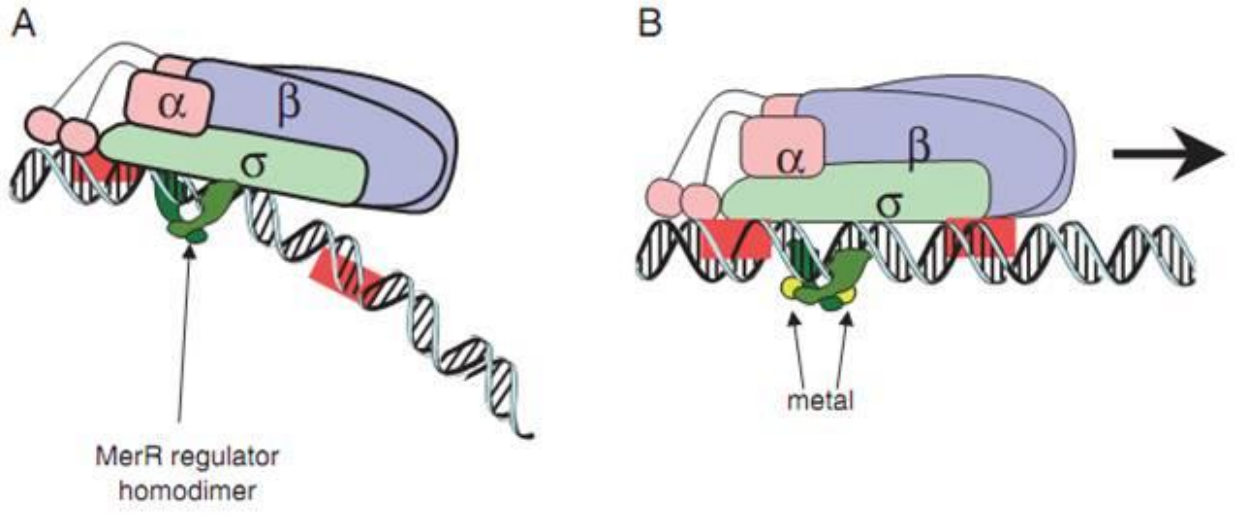
- Detects Pb^{2+}
- Constitutive expression of **lead binding protein** (BBa_I721002) by BBa_J23119 promoter
- Pb^{2+} forms a dimer with the lead binding protein that binds to the **lead promoter** (Bba_I721004)
- Lead promoter binding results in GFP expression → signal

Lead sensory construct



Mercury promoter **PmerT** (BBa_K346002) and transcription factor **MerR** (BBa_K346001)

- Constitutive production of **MerR**
- **MerR** behaves as transcription factor to **PmerT** promoter sequence
- **MerR** is only active when mercury is present
- Activation of **MerR** results in RFP expression



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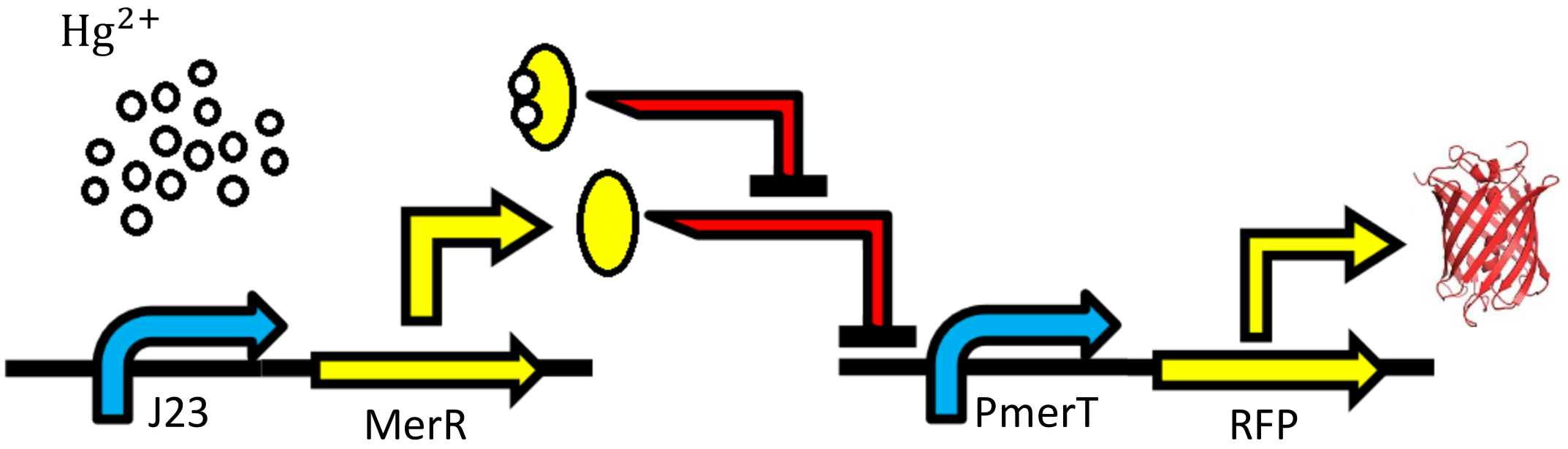
Mercury sensory construct



Reporter genes GFP & RFP

Fluorophores

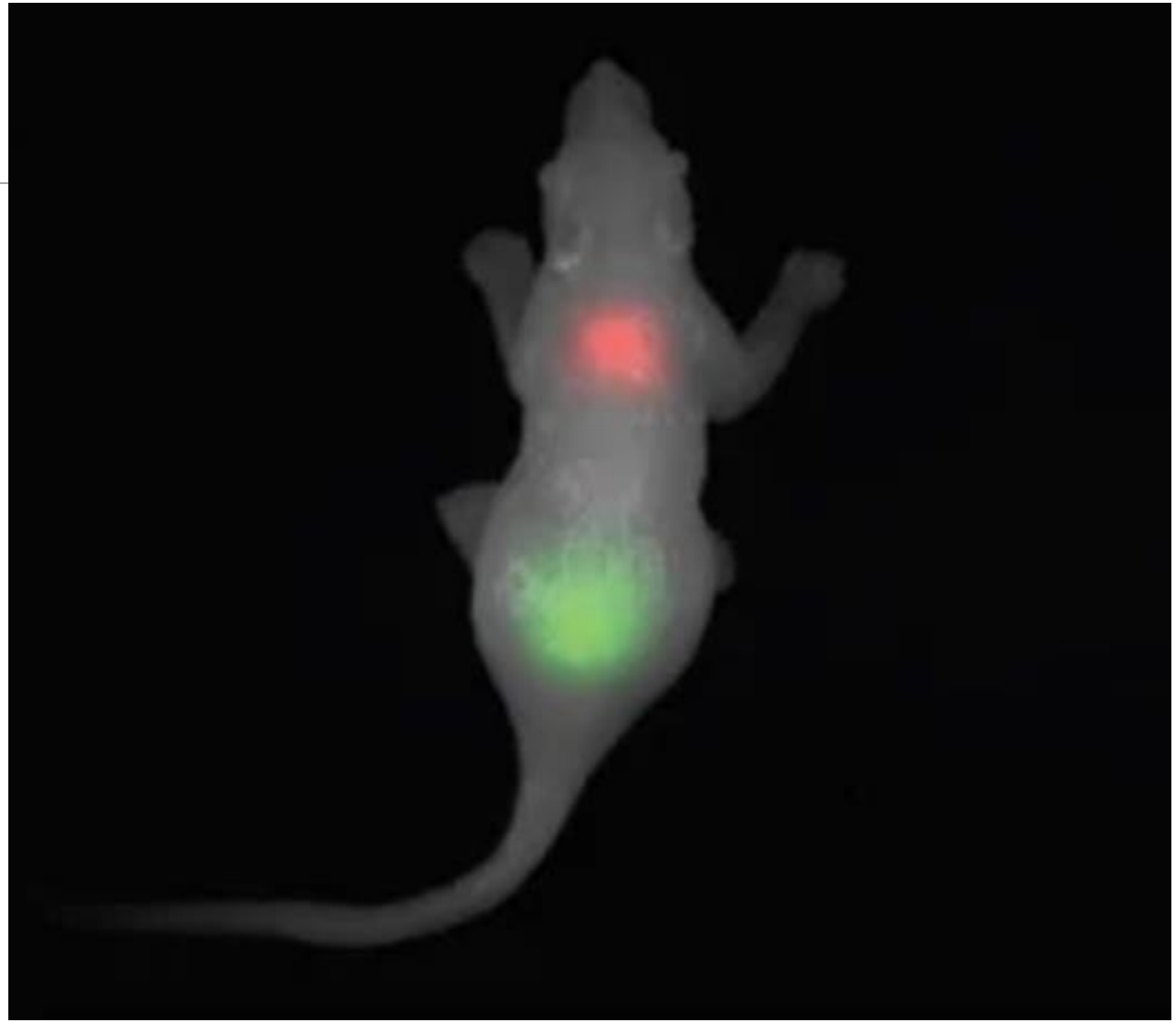
- Emits light upon light irradiation
- Widespread use after first isolation of GFP from *Aequorea Victoria*

Used for labeling and detection

RFP and GFP : Overlapping excitation range (460-520nm)

Detection: Fluorescence microscopy / flow cytometry

Flow cytometry → better for detecting overlapping signals

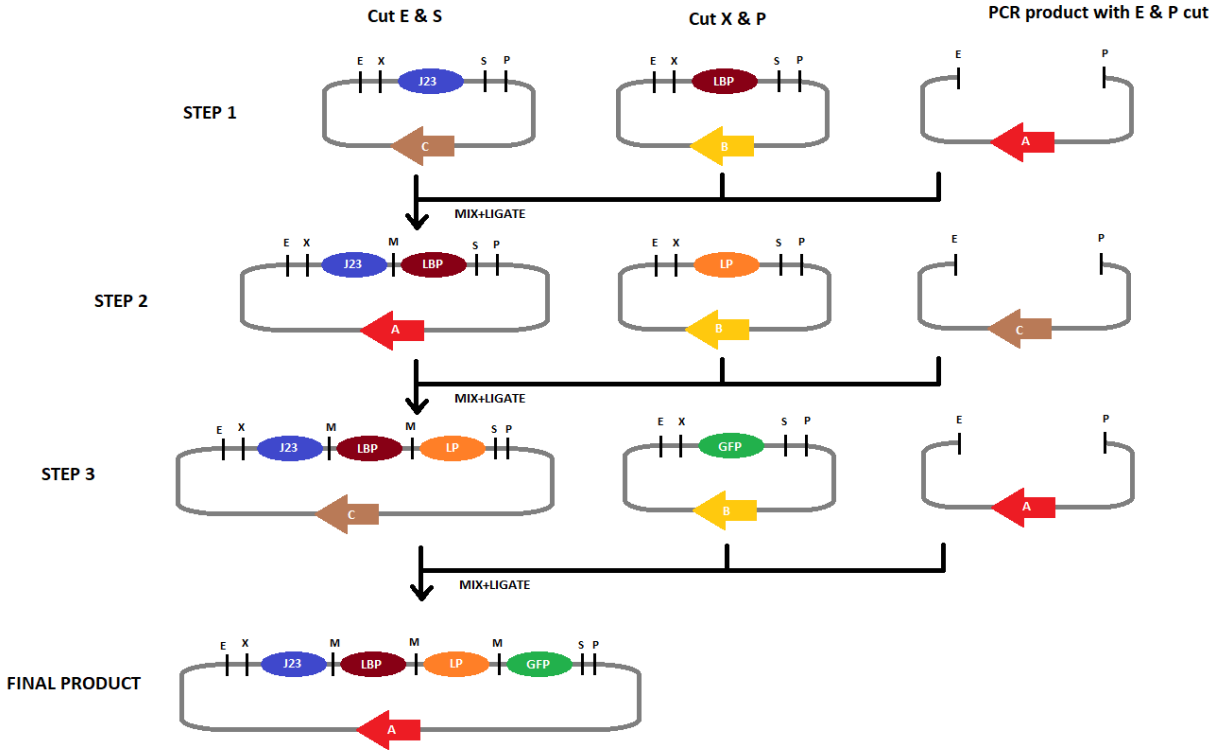


https://doi.org/10.2144/01305rr01

Picture: Analytik Jena US. 2020. *In Vivo Fluorescence Imaging with GFP and RFP*. News-Medical, viewed 23 March 2022, https://www.news-medical.net/whitepaper/20200930/In-Vivo-Fluorescence-Imaging-with-GFP-and-RFP.aspx.

Sensor assembly and truth table

Assembly example: Lead sensor plasmid (according to 3A Assembly method)

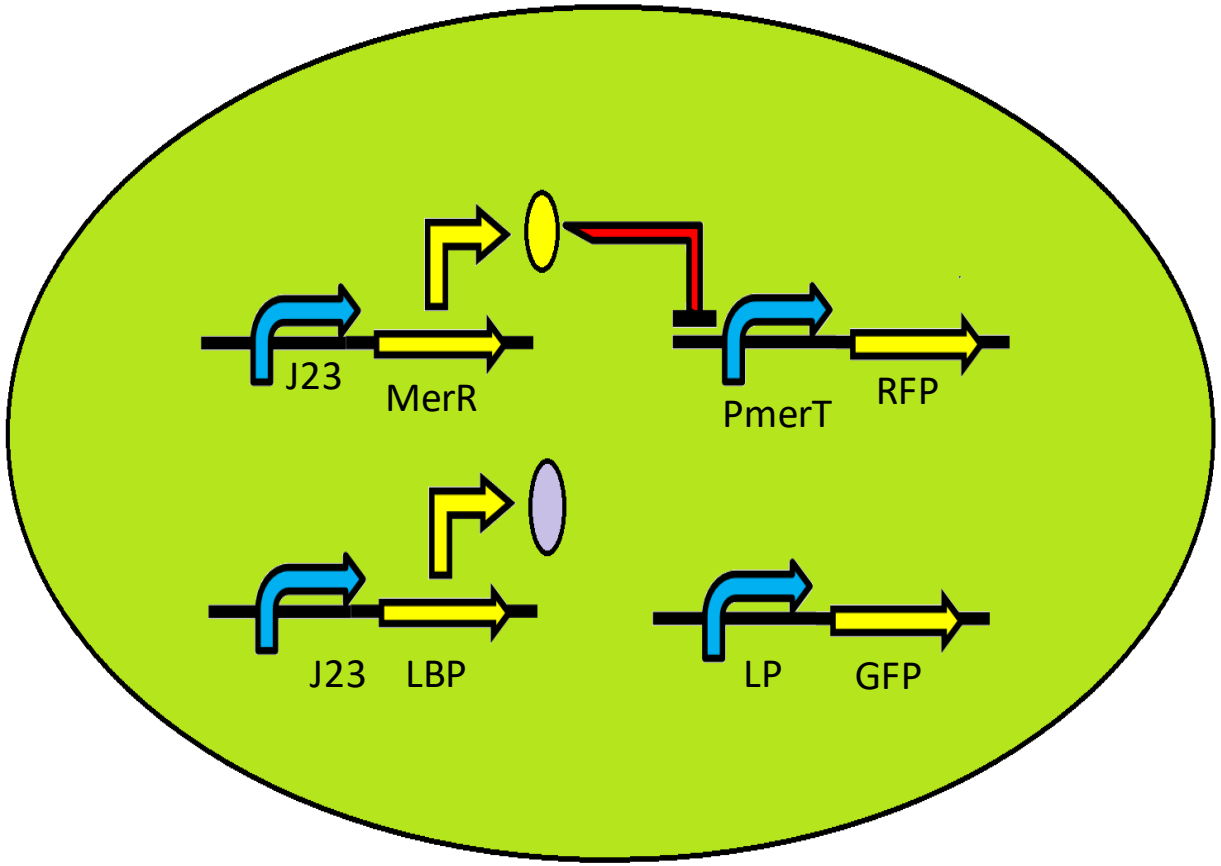


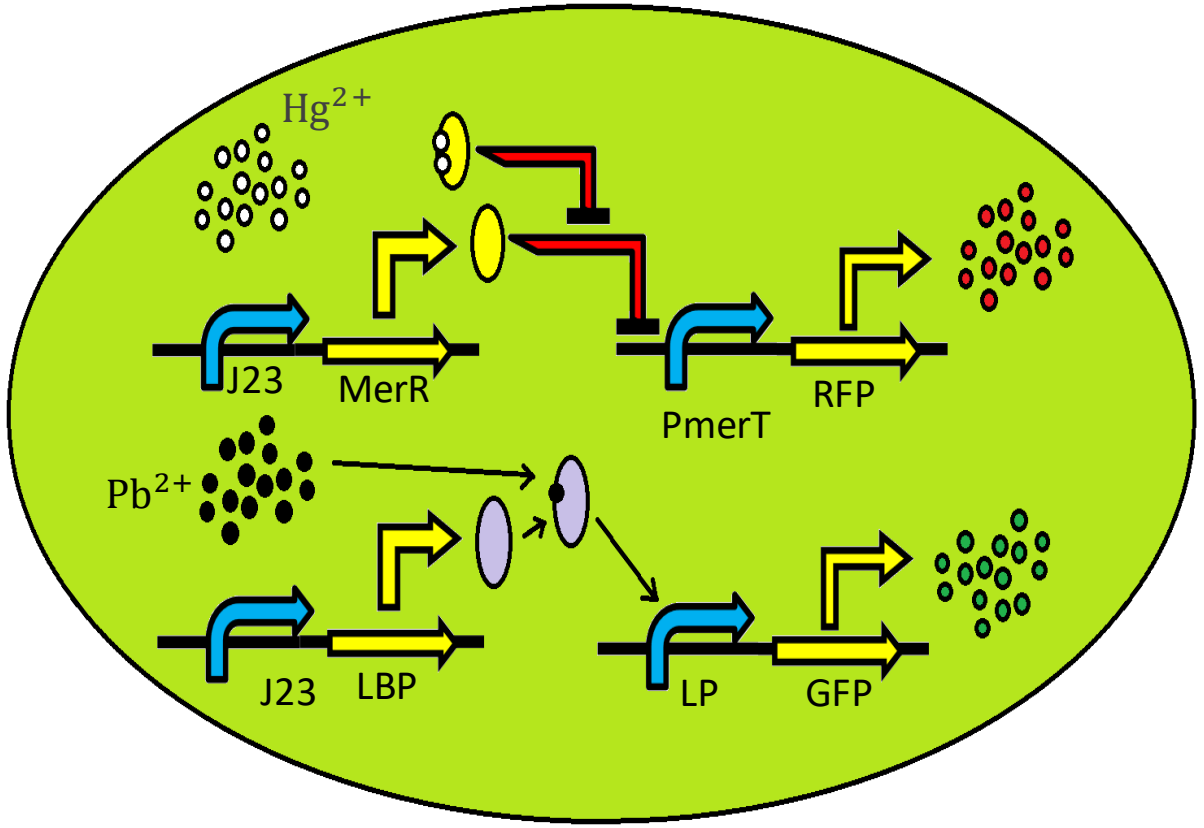
Truth table of the system:

Lead	Mercury	GFP	RFP
1	0	1	0
1	1	1	1
0	1	0	1
0	0	0	0

E = EcoRI, X = XbaI, S = SpeI,
P = PstI, M = mixed site

System off/on states





Conclusions

- The lead sensor parts are not experimentally known to function
- Detectable concentration of each metal:
  - Each sensor in its own plasmid: possible to adjust the detection limit for each metal separately
  - The tolerated level of metal contamination is site-specific
  - Detects only the ionic species Pb^{2+} and Hg^{2+}, and therefore might underestimate the true concentration of the metal
- The system would incorporate two separate plasmids, which both require their own constant selection pressure
- Detection of the signal (fluorescence) may present problems if the signal is not adequate due to expression related reasons
- At least due to reasons above, changes may need to be made after experimental testing
- The system is better for qualitative experiments due to several variables impacting the signal intensity