Lead & Cadmium Detecting Sensor

Standardization, biobricks and chassis

Heidi Leppänen, Saana Rekinen, Ronja Haikarainen and Nea Virta

Aalto University
School of Chemical Engineering

Lead & Cadmium

- Occur in trace amounts naturally in bedrock, soil, and organisms
- Released into water as a result of both natural processes and human activities, such as:
  - Coal burning
  - Heavy fuel oil (HFO)
  - Waste
- Tend to accumulate in organisms → the concentrations increase towards higher levels along food chains

Escherichia coli as the system of choice

- Aim: to construct the sensor using only the existing parts in the iGEM Registry
  - Most documented parts are for *E. coli*
- The “workhorse” of synthetic biology
- *E. coli* has transporter encoded by gene ZntA which exports Zn/Pb/Cd out of the cell

  → Assumption that we are working with a ZntA deletion strain

Overview of the Designed Sensor

Can be divided into 3 devises:

1. **Detects Lead**

- Requires a lead-binding protein and lead
- Output is a blue fluorescent signal

2. Detects Cadmium

- Requires only cadmium
- Output is a red fluorescent signal

3. Is dependent on the first two

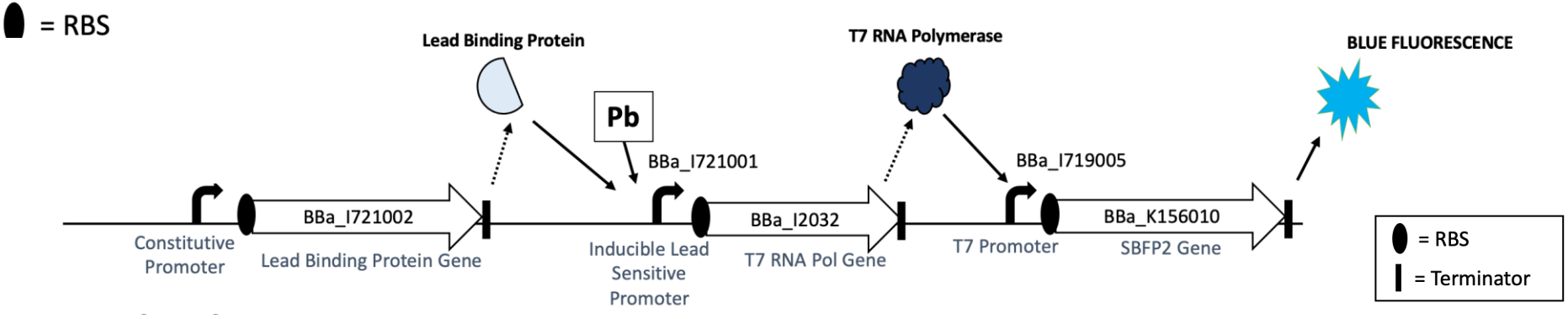
- In the presence of both lead and cadmium
    - AND gate
- Output is a yellow fluorescent signal

INPUTS		OUTPUTS		
Pb	Cd	SBFP2	mCherry	EYFP
0	0	0	0	0
1	0	1	0	0
0	1	0	1	0
1	1	1	1	1

In the Presence of Lead...

- Lead binding protein
  - Constitutive promoter
- Together, the lead binding protein and lead ions induce the lead promoter (AND GATE)
  - T7 RNA Pol gene
    - T7 RNA Polymerase induces T7 promoter
      - SBFP2 gene → Blue fluorescent signal

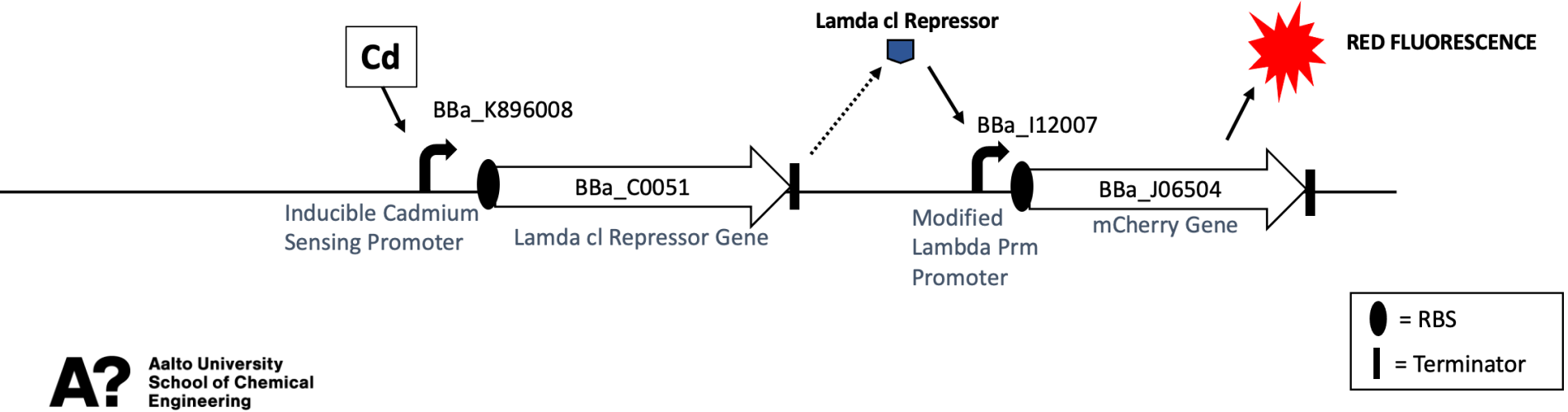
- T7 RNA Polymerase induces T7 promoter in the system detecting both lead and cadmium



In the Presence of Cadmium...

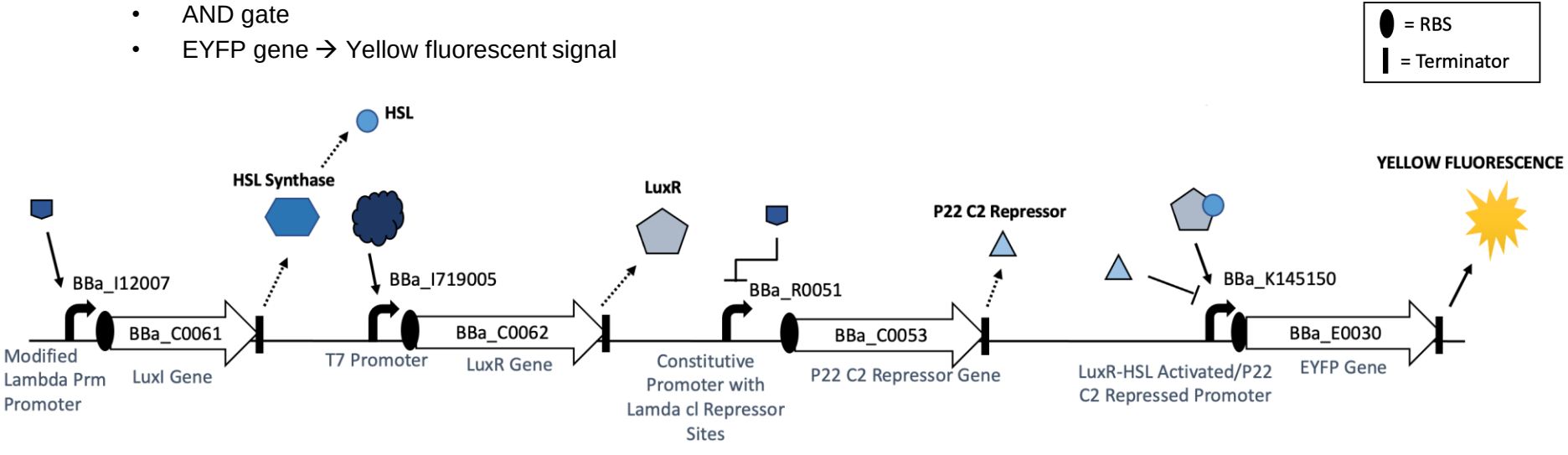
- Cadmium ions induce the cadmium promoter
  - Lamda cl Repressor gene
    - Lambda cl repressor activates a modified promoter
      - mCherry gene → Red fluorescent signal

- Lambda cl repressor is needed when detecting both lead and cadmium



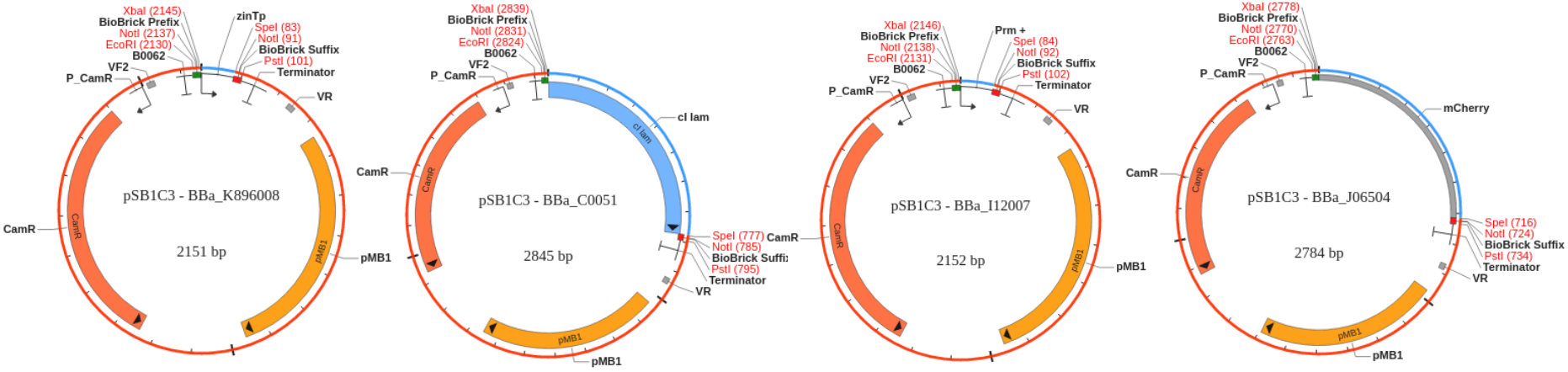
In the Presence of Both Heavy Metals

- Lamda cl repressor induces a modified Lambda Prm promoter
  - LuxI gene → HSL (signalling molecule)
- T7 RNA polymerase induces T7 promoter
  - LuxR gene
  - LuxR and HSL form a complex
- Lamda cl repressor represses a constutitive Pr promoter with Lamda cl repressor sites
  - Constutively produced P22 C2 repressor inhibits the hybrid promoter
- Hybrid promoter
  - LuxR-HSL complex activates and P22 C2 represses
  - The promoter has a very low background activity
  - AND gate
  - EYFP gene → Yellow fluorescent signal



Measuring Fluorescence Signals

- Selected Fluorophores with non-overlapping emission spectra
- mCherry (BBa_J06504), EYFP (BBa_E0030), and SBFP2 (BBa_K156010)



Assembly of the cadmium responsing system

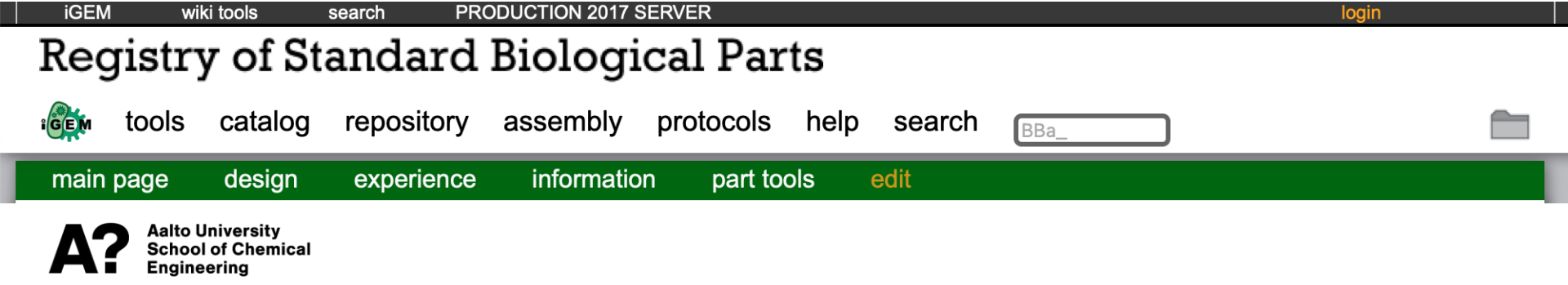
- The genes for the system responding to Cd are found in the plasmids shown above (iGEM registry)
- The assembly can be performed with the BioBrick RFC10-standard:

5' - GAATTC GCGGCCGC T TCTAGA G ... T ACTAGT A GCGGCCG CTGCAG - 3'

EcoRI NotI XbaI SpeI NotI PstI

Conclusions & Our Experience with BioBricks

- Many parts for many purposes
- Our design process was heavily affected by what was NOT available
- Standardized parts but non-standardized annotations...
- Catalog is missing a proper search/filter function



Thank you for your attention!
Any questions?

References

Surette, M. G., Miller, M. B., & Bassler, B. L. (1999). Quorum sensing in Escherichia coli, Salmonella typhimurium, and Vibrio harveyi: a new family of genes responsible for autoinducer production. *Proceedings of the National Academy of Sciences of the United States of America*, *96*(4), 1639–1644. https://doi.org/10.1073/pnas.96.4.1639

iGEM Organization. (n.d.). *iGEM Registry of Standard Biological Parts*. Retrieved March 24, 2022, from http://parts.igem.org/Main_Page