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Cd^{2+} Sensing System from Biobricks

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CHEM-E8125 Synthetic Biology

Motivation

- **Cadmium**
 - *is a heavy metal*
 - *is highly toxic*
 - *bioaccumulates*
- **Human exposure via**
 - *tobacco*
 - *food*
 - *drinking water*

Our solution

- *system for detecting Cd^{2+} from soil or water*
- *input: Cd^{2+}*
- *output: GFP*
- *Cd^{2+} inducible promoter CadA (K1724000)*
 - *activated when $[\text{Cd}^{2+}] > 3 \cdot 10^{-8} \text{ M}$*
 - *specific*
- *cascade to generate ultrasensitive response*

Biobricks used

Promoters

- Constitutive Anderson promoter (BBa_K823004)
- CadA repressible promoter (BBa_1724000)
- PenI regulated promoter (BBa_R0074)
- pTet repressible promoter (BBa_R0040)

Terminators and RBS

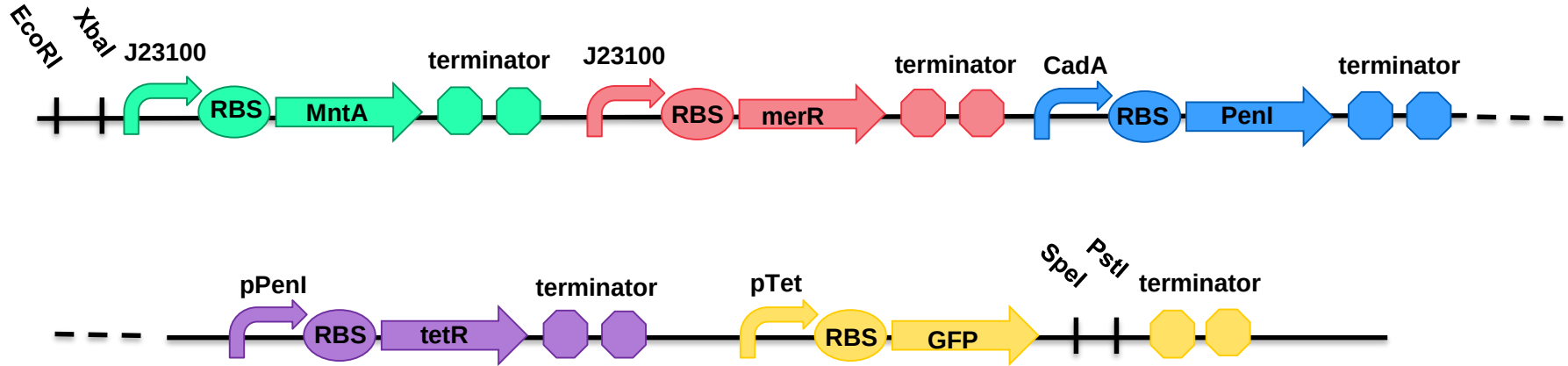
- Double terminator (BBa_B0015)

- RBS (BBa_B0034)

Coding regions

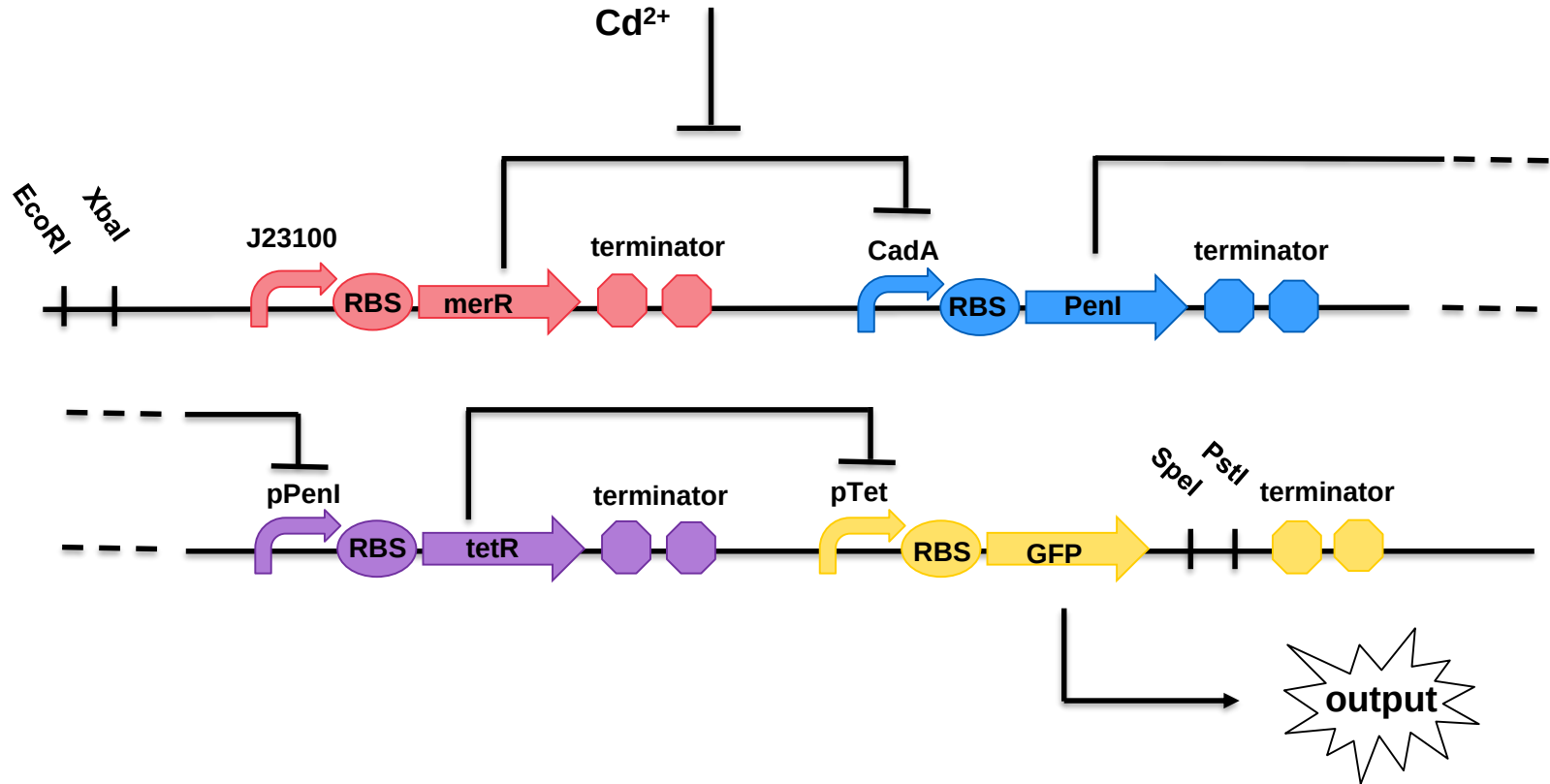
- Cd²⁺ and Mn²⁺ transporter with RBS (BBa_K1724003)
- merR with RBS (BBa_K1724002)
- PenI with LVA tag (BBa_C0074)
- tetR with RBS and LVA tag (BBa_K1520505)
- GFP expression cassette (BBa_I13504) with RBS and double terminator in pSB1A3 plasmid

Assembly standard RFC [10]

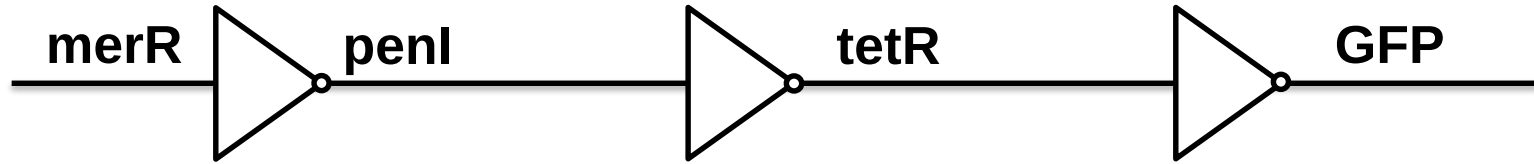


- **CamR resistance marker + promoter**
- **pMB1 origin of replication**

Repression cascade concept

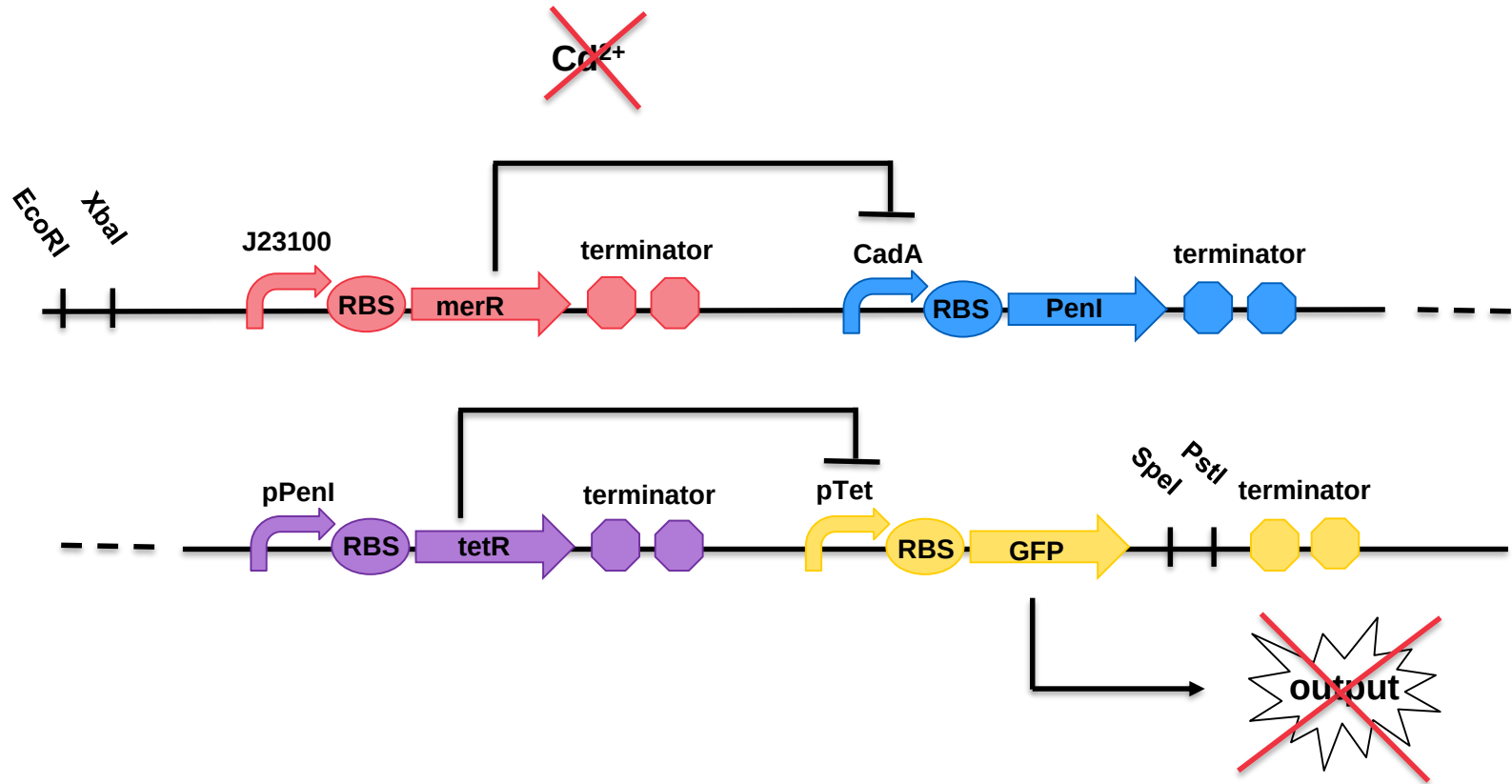


Series of NOT gates



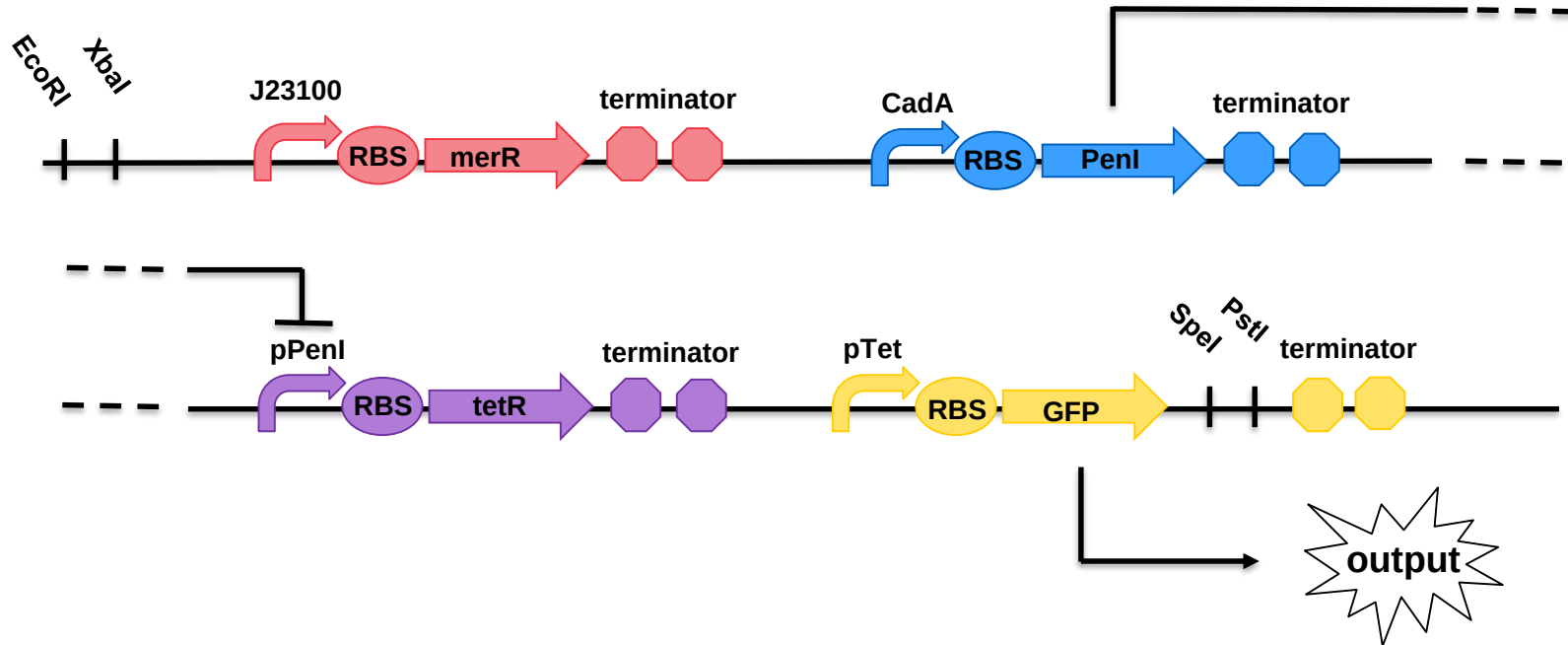
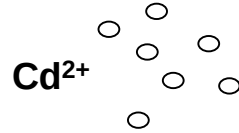
Cd^{2+}	merR	penI	tetR	GFP
1	0	1	0	1
0	1	0	1	0

In off-state

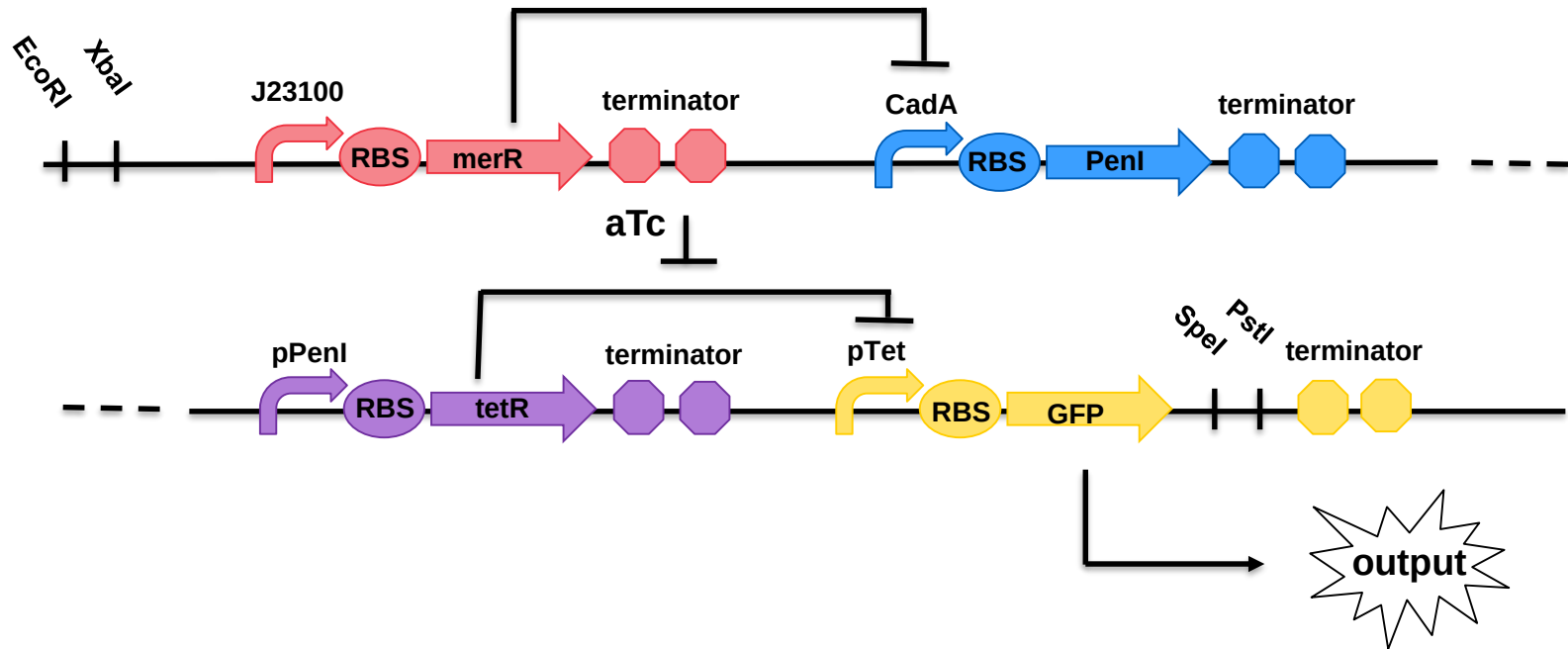


In on-state

Cd^{2+} inactivates the merR-protein $\rightarrow$ inhibits the repression of the CadA promoter



Induction by anhydrotetracycline



References

https://www.who.int/ifcs/documents/forums/forum5/nmr_cadmium.pdf
(07.03.2019)

Hooshangi, S., Thiberge, S., and Weiss, R. Ultrasensitivity and noise propagation in a synthetic transcriptional cascade. PNAS March 8, 2005 102 (10) 3581-3586. <https://doi.org/10.1073/pnas.0408507102>
(06.03.2019)

www.parts.igem.org (06.03.2019)

Thank You!