## Component Justification and description Table1: Components

CI promoter for RcnA	CI promoter for RcnA pcl is repressed by CI and this way, we can repress the RcnA transcription.
RcnA <sup>1,2</sup>	Nickel and cobalt efflux pump protein. We chose RcnA due to its high especificity for nickel. Besides, it is encoded by only one quite short gene. Nickel was
	selected because E. coli can tolerate high doses of this metal, and the amount the cell uses is negligible compared to the concentrations we are using.
BBB1MCS 53	It is a gentamicin resistant low copy number plasmid. Belongs to a compatibility group different from the PRK415's one

AHL<sup>4</sup> pBBR1MCS-5 Acyl-homoserine lactone. Is the input signal. We decided to use this compound because its easy to degradate it and start a new signal icis a germanincin resistant low copy hamber prasmia: belongs to a companionly group aniere in home in 1974 to a one

TetR promoter for luxR <sup>5</sup> A moderate-strong promoter for luxR, it ensures that LuxR's concentration is not the limiting step for CI synthesis. Thus, we control all CI expression through

the addition of AHL

LuxR<sup>6,7,8</sup> LuxR binds to AHL to activate the transcription of the genes downstream of LuxR:AHL promoter

LuxR:AHL promoter Inducible by the AHL:LuxR dimer

CI\*9,10,11,12 CI\*1.2 is a modified form of the cI repressor from lambda phage. It has a LVA tail for quick degradation. A quicker CI degradation would ensure the signal is not

lacZ promoter 13 Constitutive promoter, allowing an active aiiA transcription so AHL can be efficiently degraded always on

E. coli W3110 Yoh-

E. coli k12 substrain W3110 which has a mutation in the YohM (RcnA) gene. YohM was replaced for kanamycin resistance

pRK415 16

It is a tetracycline resistant low copy numer plasmid which carries LacZ gene

|AiiA is the protein which degradates AHL ensuring that the signal doesn't last for too long

(a higher amount of AiiA means a quicker AHL degradation and could lead to signal weakening

pLacZ is a mutated version of pLacZ, produced by site directed mutagenesis, changing a T for a C. This was done in the pursuit to lower aiiA's transcription,

AiiA 14,15

lacZ\* promoter