E.colock

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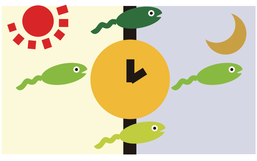
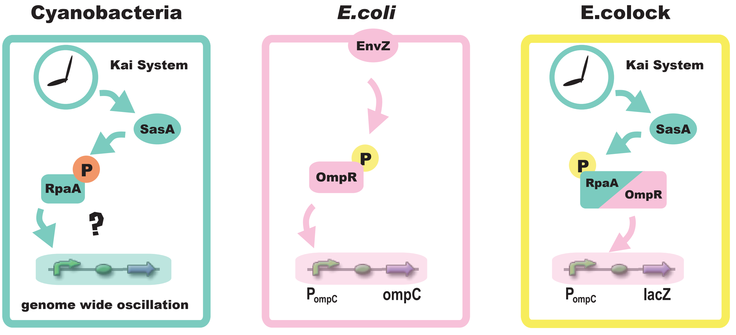
Goal ~ Self-cycling-E.coli



We aimed to install **Kai system** to E.coli. Kai is a self-sustainable oscillation system to globally control the protein expression in cyanobacteria. Kai genes should be installable, but we need to make sure we can pick up the Kai cycle and read it out as color change.

Background



Kai SYSTEM

Though Kai system is well characterized in its own, there are missing links between kai cycle and the expression controls two below.

SasA is histidne kinase (HK) thought to connect between Kai Oscillation and transcription.

(H.Iwasaki et al., *Cell*, **101**: 223-233, 2000)

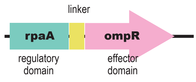
RpaA is a cognate response regulater (RR) required for translating Kai cycle to genome-wide expression pattern of cyanobacterial genes.

(N.Takai et al., *PNAS*, 2006, **103** : 12109-12114)

E.colock Project

SasA and RpaA is highly homologous to EnvZ and OmpR, respectively.

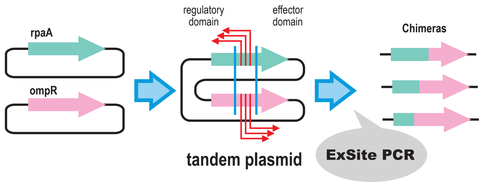
We attempted to make a series of chimeras between RpaA (reguratory domain) and OmpR (DNA binding domain), hoping for the one that can well pick up the Kai pulse via SasA and activate OmpC promotor.



Experiment

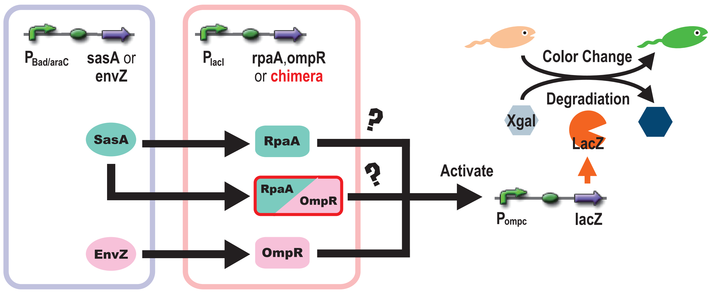
CHIMERA DESIGN

We tried to create 36 chimeras gene of rpaA-ompR and gained some of them. There are differences in cutting point, but all of them have recerver domein of RpaA to communicate SasA, and DNA-binding domain of OmpR to bind and activate OmpC promoter.



OUTPUT DESIGN

Run the systems we tested various available promotors for expressing chimeras, among them we effectively liked pBad/AraC promoters.
As an output signal, we've chosen lacZ activity which can easily visalized on X-gal of plates. We used ΔEnvZ strains that ha s [P_{ompC}-lacZ] in its genome.



Next Step

We could clone some chimeras, but still haven't checked their functions yet. The next step is to find the functioning ones from the chimera libraries.