Tianjin University 2008 iGEM

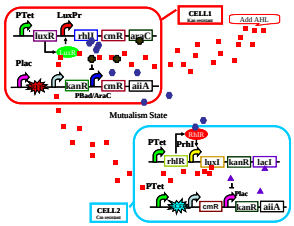
Synthetic Convertible Ecosystem

Goals and Objectives

- Explore new methods for deeper understanding of the natural ecosystems and improving industrial coculture.
- Construct a synthetic ecosystem the relationship within which is artificially controllable.
- Apply Prisoners' dilemma to regulate co-cultured strains through changing internal and external factors.

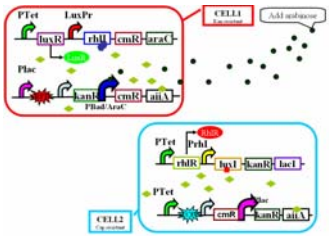
Strategy

A synthetic convertible ecosystem comprised of two strains the relationship between which could be switched between **mutualism** and **competition** has been built. The relationship between the two could be regulated through altering controllable factors, among which are environmental pressure, internal communication, external signals.

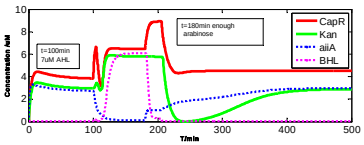


The communication between the two strains was realized by quorum sensing mechanisms, while a "toggle switch" was built to regulate the relationship between the two.

As in Prisoners' Dilemma, the partners in this game, the co-cultured strains will take a friend/mutualism strategy when they could communicate with each other, and they will take a foe/competition strategy when the communication is interrupted.



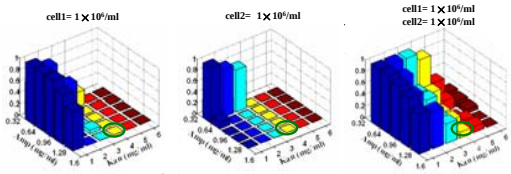
Modeling and Simulation



The viability of mutualism and competition varies in different conditions, the adding of AHL, after a short time of perturbation, leads to a mutualism phase, where both strains' growths increased to a higher level, while the adding of arabinose resulted in the significant decline of one of the strains.

Experimental Results

We have tested our already-built part of the system, from the figure below, conclusion could be drawn that obligatory symbiosis was achieved.



In the region with medium concentrations of antibiotics, which are illustrated by the green rings, the survival of one strain is the prerequisite for that of the other, thus we came to the conclusion that obligatory symbiosis is formed.

Foolproof Genetic Self-Assembly System

Goals & Objectives

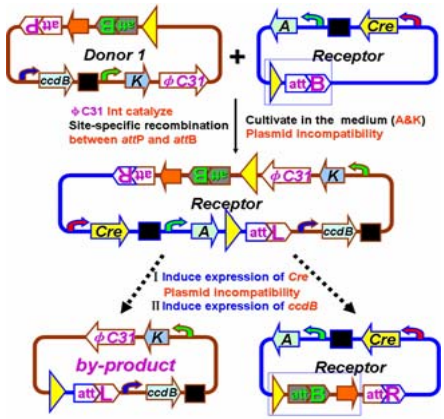
We have designed and constructed a novel foolproof genetic self-assembly system, which could assemble automatically DNA sequence *in vivo* by applying the site-specific recombination gene *attB/attP* & *loxP*. Based on the plasmid incompatibility and the killer gene *ccdB*, we could select the very vector containing the linked gene we have planed.

Introduction

It is well known that orderly assembly of genetic parts is time-consuming and expensive. In order to make synthetic biologists free from laborious and repetitive lab work , we have developed a groundbreaking method "Fool proof genetic self-assembly system.

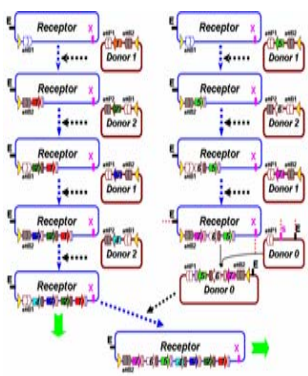
Design and Process

Design: We used *attP / attB* site-specific recombination to insert the target gene, and the *cre / loxP* system was employed to remove excess insertion sequence. After ingenious arrangements of two sets of *attP / attB* site and the location of *loxP*, the system can implement the gene insertion iteratively while delete the redundant sequence to simplify to a new receptor. The experiment was verified by the selective forces of two antibiotic gene and the killer gene ccdB which was able to kill cells containing undesired recombinant plasmids



Process: When introduced to the E.Coli, Donor vector with the desired GENE1 integrated with Receptor vector as one circular plasmid through the BP reaction. Cre protein catalyzes the recombination of two loxP sites to obtain two separate plasmids: one retaining the desired GENE1, while the other preserving a killer gene *ccdB*. The plasmids were separated into different cells as result of plasmid incompatibility. The *ccdB* gene was induced to select the target plasmid.

Highlights & Prospect



- **Preciseness:** Orderly insertions was realized through recombination between attP/attB sites.
- **Selectivity:** selective medium containing two antibiotics and plasmid incompatibility as well as killer genes ensures the selection of the desired recombinant DNA sequences.
- **Continuity:** limited times of recombination can be achieved, theoretically.
- **Time efficiency:** Reduce the time required during one round of gene cloning experiments to only two days.
- **Easy:** Standardized plasmids reduce the labor to only introducing the foreign plasmids and cultivating the cell.
- **Environmental friendly:** Less waste released by reactions in vivo.