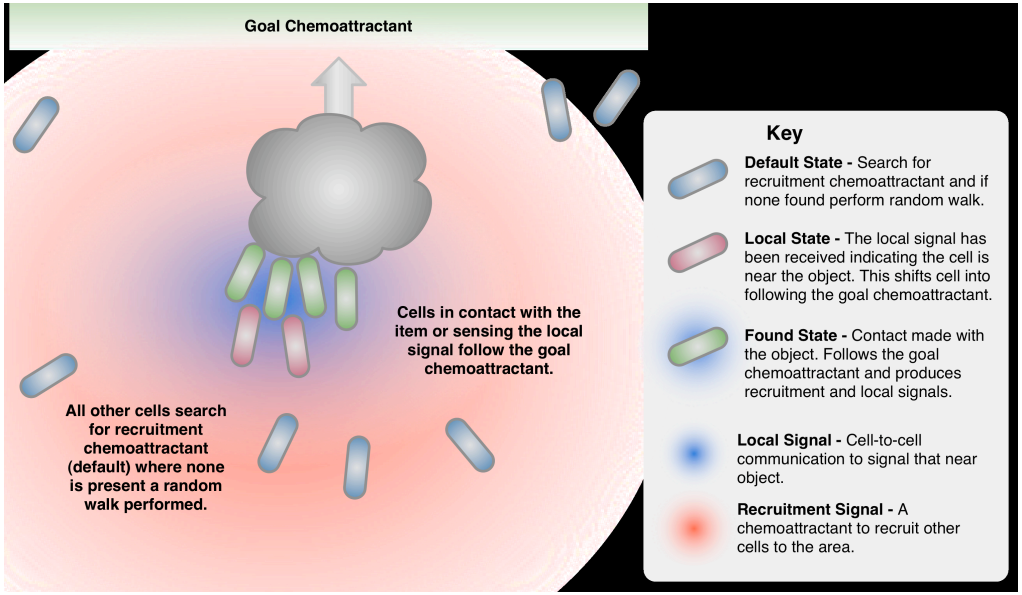
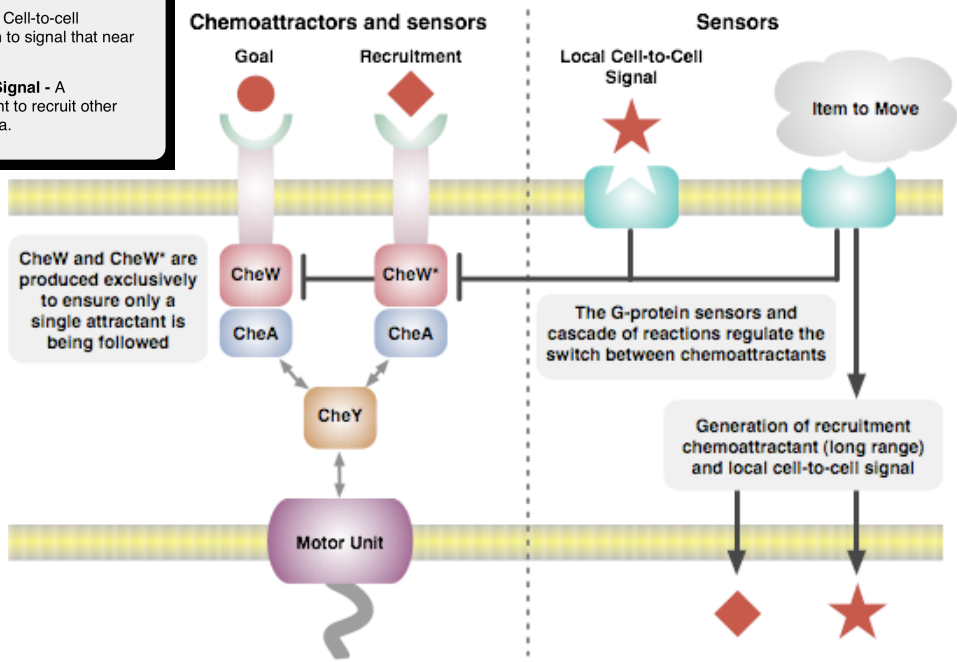
A little reminder……

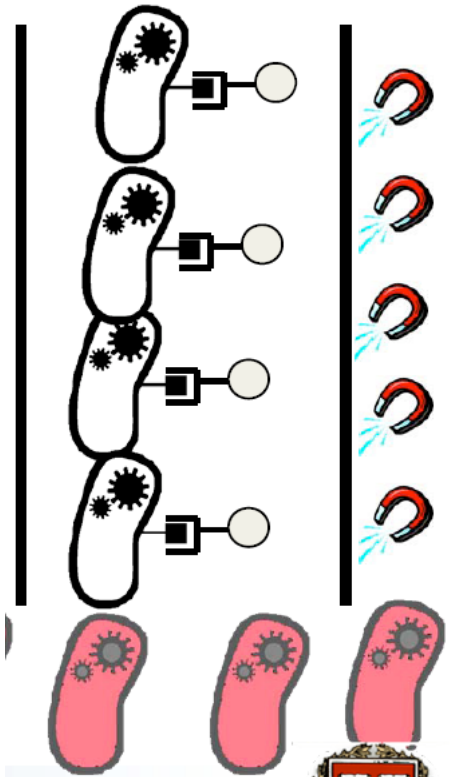




Sensing the particle

Harvard 2007- Cling.*E-coli*

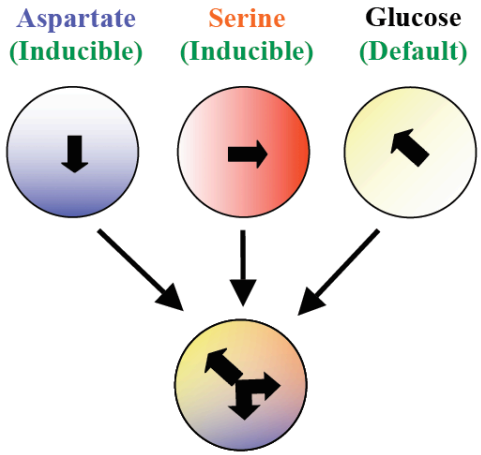
- Successfully produced functional N-terminal his/strep2-tagged AIDA1. (Biobrick no??)
- Magnetic-activated cell sorting (MACS) and Fluorescence-activated cell sorting (FACS) assays
- Binding with high affinity to bead might affect motility??

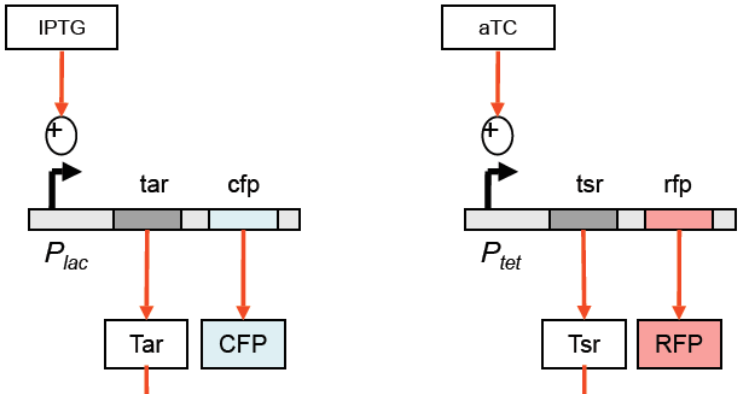


Chemotaxis

Bangalore 2006 – X-Y chemotaxis

- 2D control of chemotaxis on tri-gradient setup.
- Produced three biobricks that might be useful in our project:
  - Plac – tar – CFP (J22001)
  - Ptet – tsr – RFP (J22005)
  - Plac – tar – CFP - Ptet – tsr – RFP (J22010)

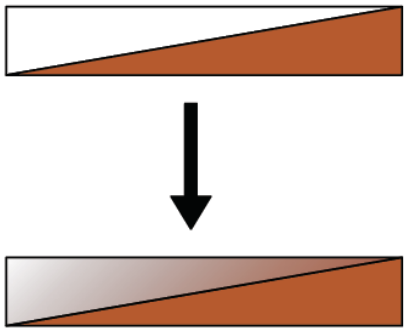


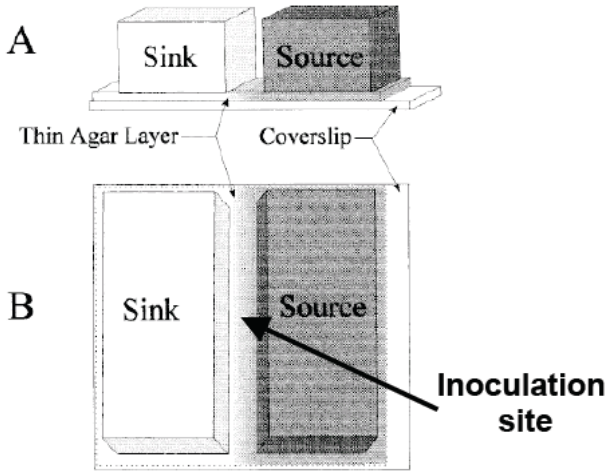


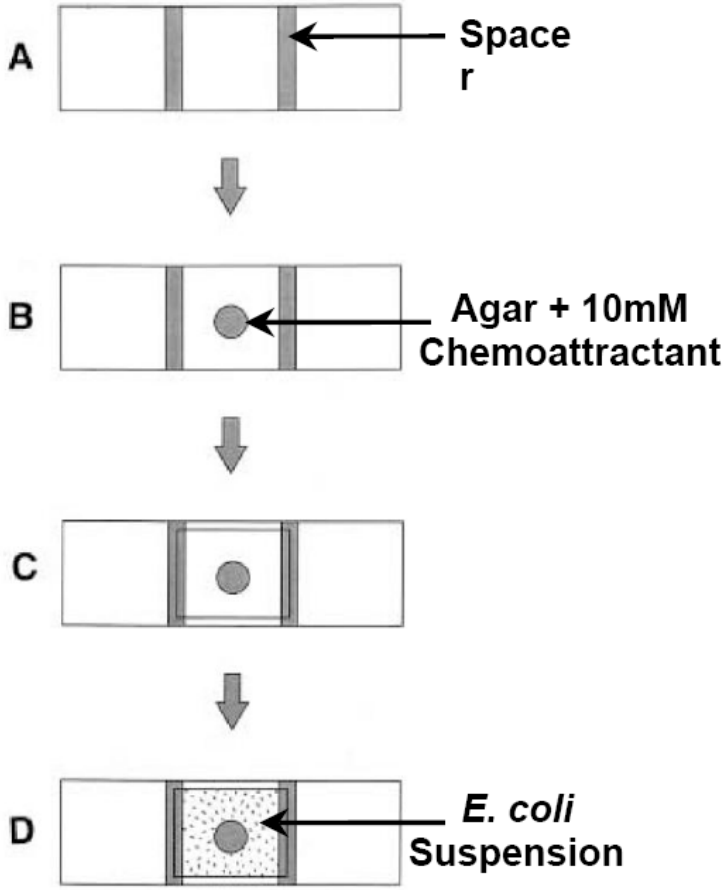
Chemotaxis

Bangalore 2006 – X-Y chemotaxis

- Chemotaxis assays:
  1. Slant plates
  2. Bridge setup
  3. Plug assay



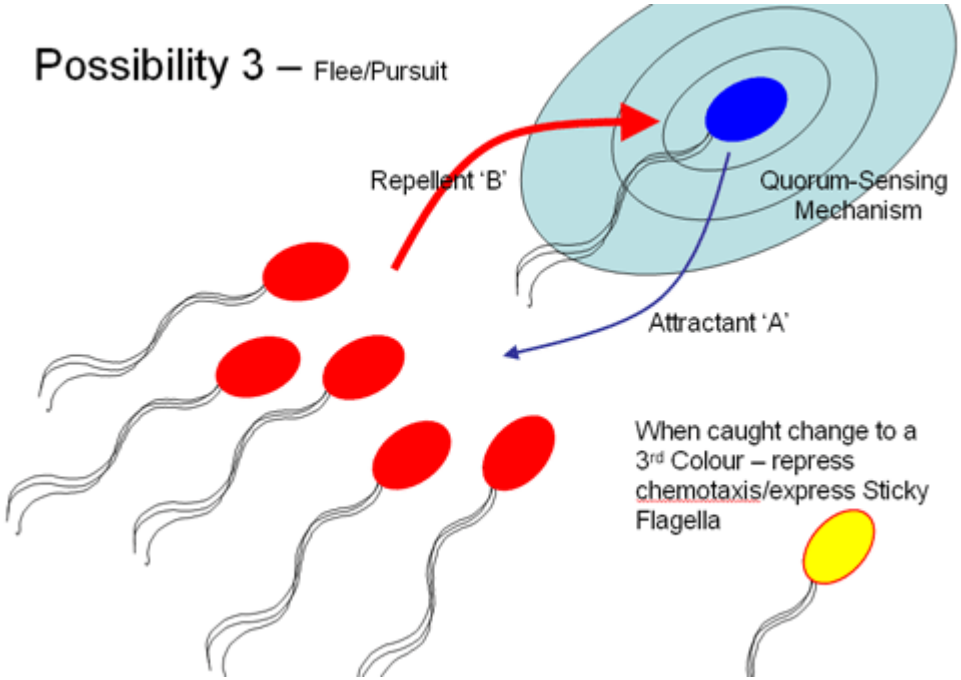




Chemotaxis

Cambridge 2005

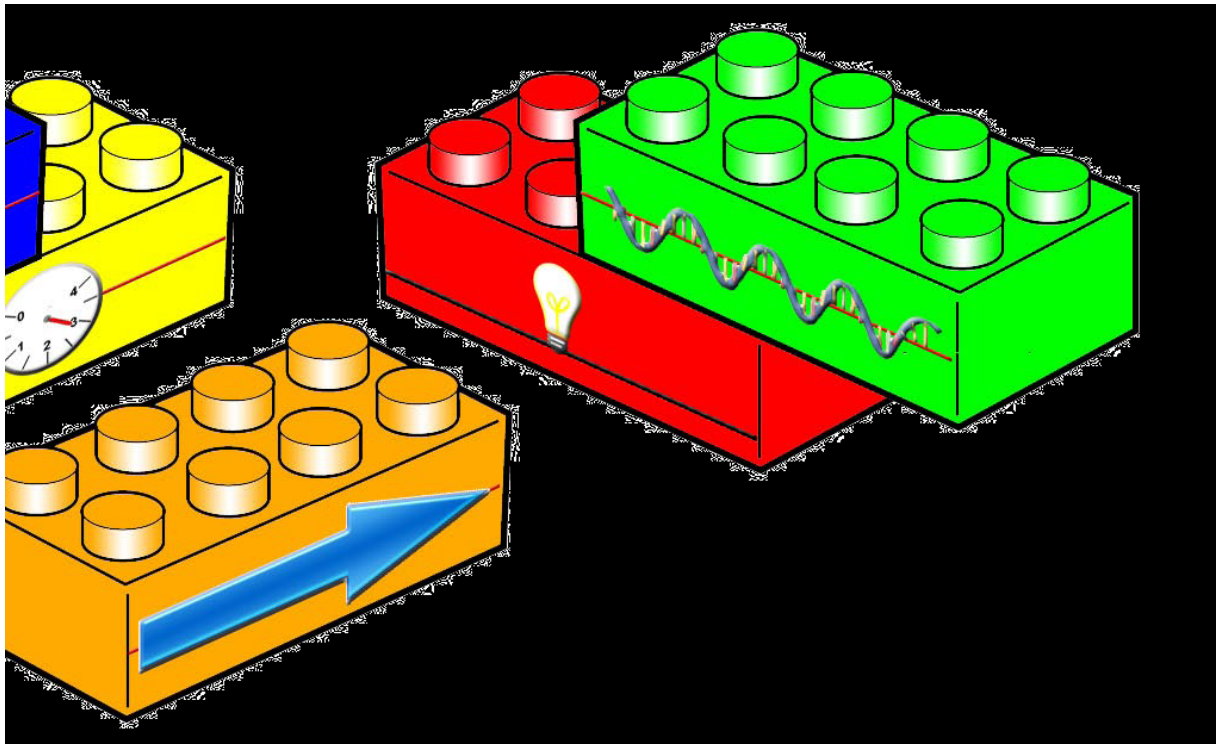
Some sort of chemotaxis, on and off but think is predetermined, v little info on wiki, most documentation missing or inadequate



Chemotaxis

Cambridge 2005

Results not great

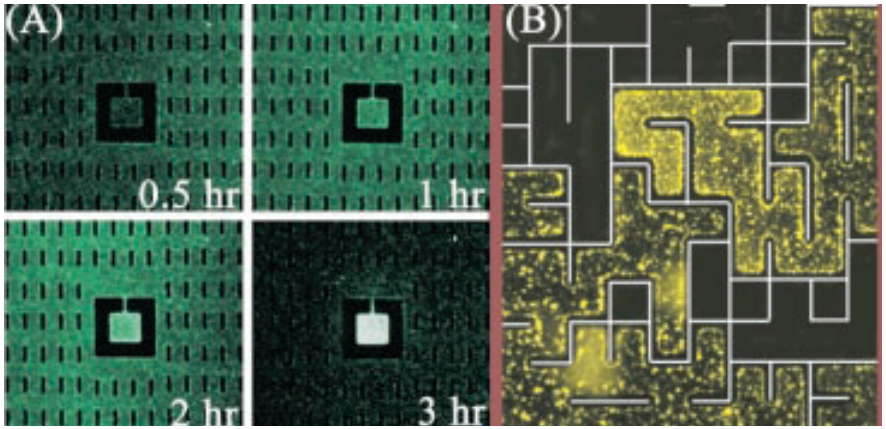


http://www.ccbi.cam.ac.uk/iGEM2005/index.php/Main_Page

Recruitment

Park *et al*. (2003) Motion to form a quorum. *Science.* **301**, 188.

- Showed that E.coli and V.harveyi can use chemotaxis to form a quorum in confined spaces.
- “Cells accumulate in the enclosure because they are attracted to each other due to their secretion of amino acids, such as glycine, that are chemoattractants”.
- “In nutrient-depleted environments…the cells themselves become sources of attractant molecules.”



Alternatives…

Using default glucose gradient (Bangalore 2006) that guides bacteria from inoculation site towards the particle????

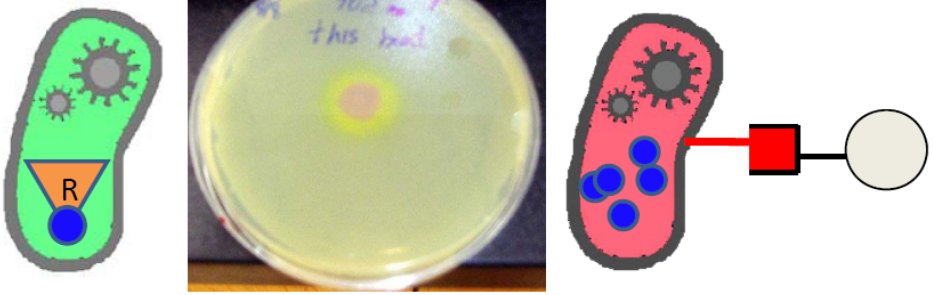
Quorum Sensing

Havard 2007 – Quorum sensing

- Prepared ‘Receiver’ (luxR-GFP) (T9002) cells and Co-transformed ‘Sender’ cells (luxI-RFP/AIDA-strep2) (S03623 + I13507 / ??)
- Demonstrated that co-transformed sender cells accummulate around streptavidin beads and that ‘receiver’ cells are able to detect quorum signal released by ‘sender’ cells.
- Binding to the bead however, is not an signal for production of the quorum signal.

Sender

Strep2 tag



Receiver

Quorum Sensing

UT Austin – Quorum sensing

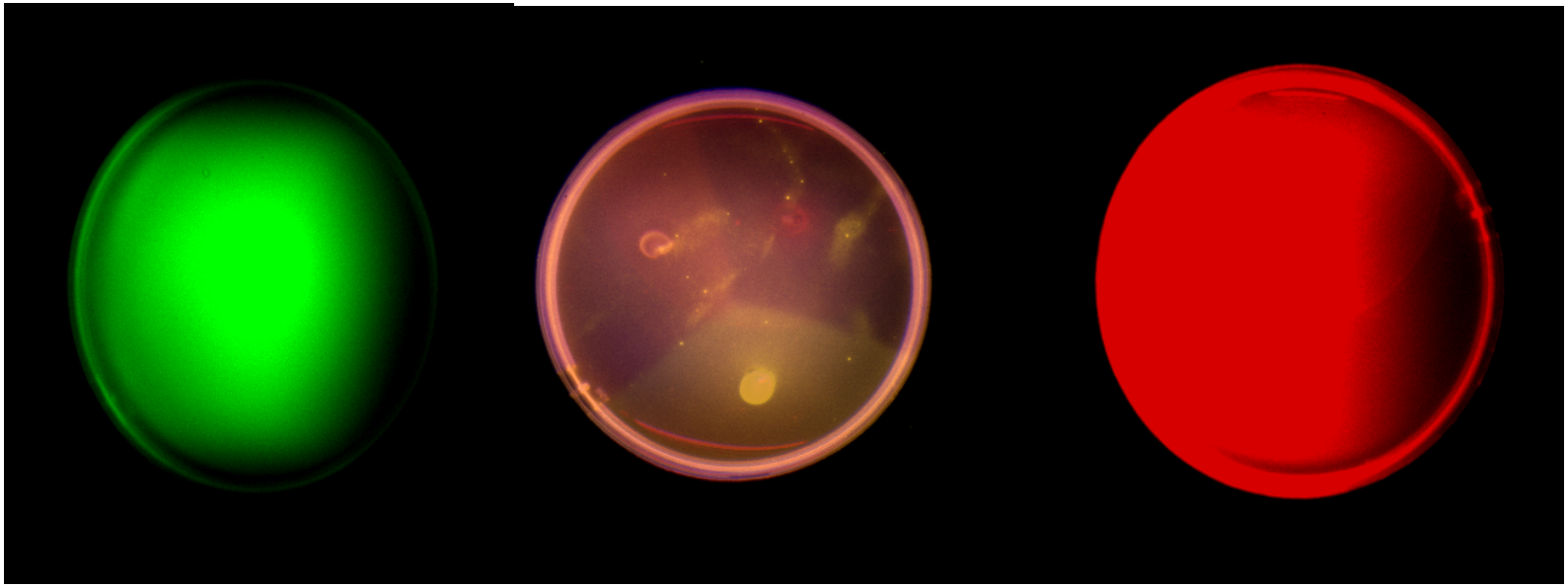
- *E. coli* carried 3OC6HSL amplifier and a pseudomonas autoinducer (AI-1) amplifier
- HSL activates high level production of HSL
- PAI activates high level production PAI
- Both compounds diffuse out of cells in all directions

- *Also used bio brick to repress production of both HSL and PAI in 660nm light*
- *allowed cells in a particle area to be targeted by using light (or the lack of light)*

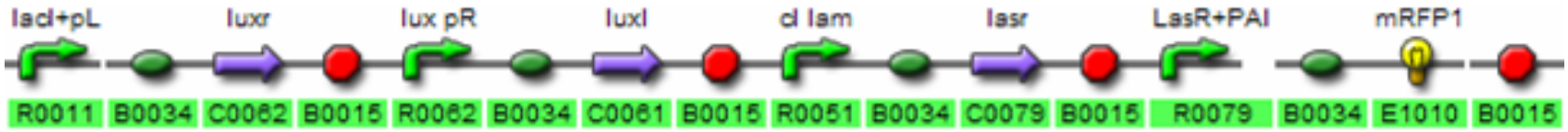
Quorum Sensing and the chemotactic switch

Cambridge 2006

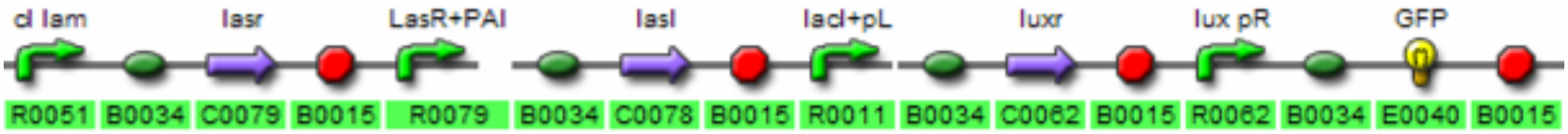
- Aiming for pattern of Ecoli of 2 types, one expressing RFP, the other GFP
- When there is a high concentration of one type in an area the other type will change into the 1st type



- Used 2 types of homeserine lactone (HSL) C6 and C12. Used in Quorum sensing
- One type produces C6 HSL and responds to C12 HSL,



• the other type Produces C12 and responds to C6 that has been produced by the 1st type



•Using a series of operons had 2 final types glowing either red or green that both inhibited and promoted each others cell types.

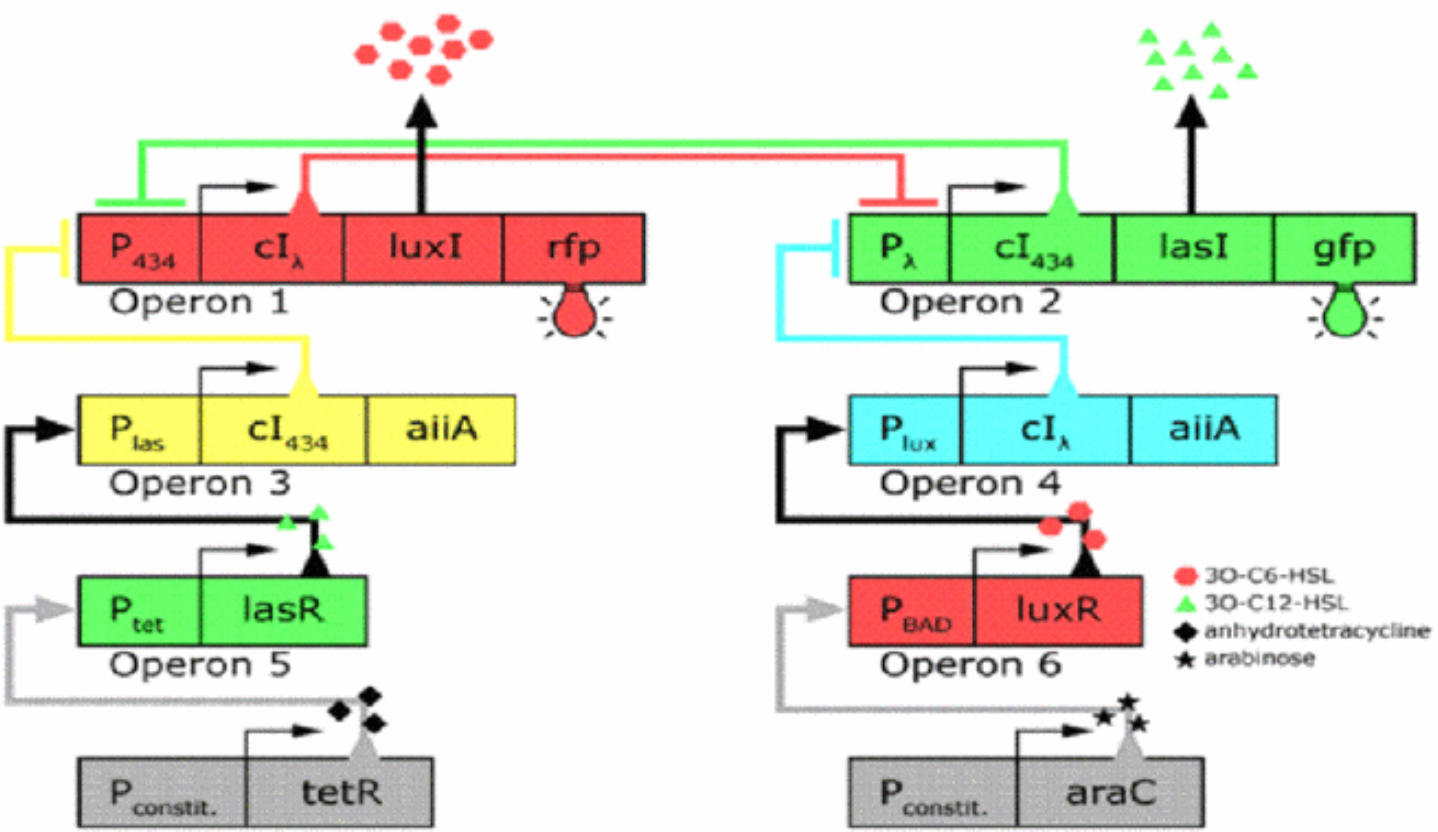
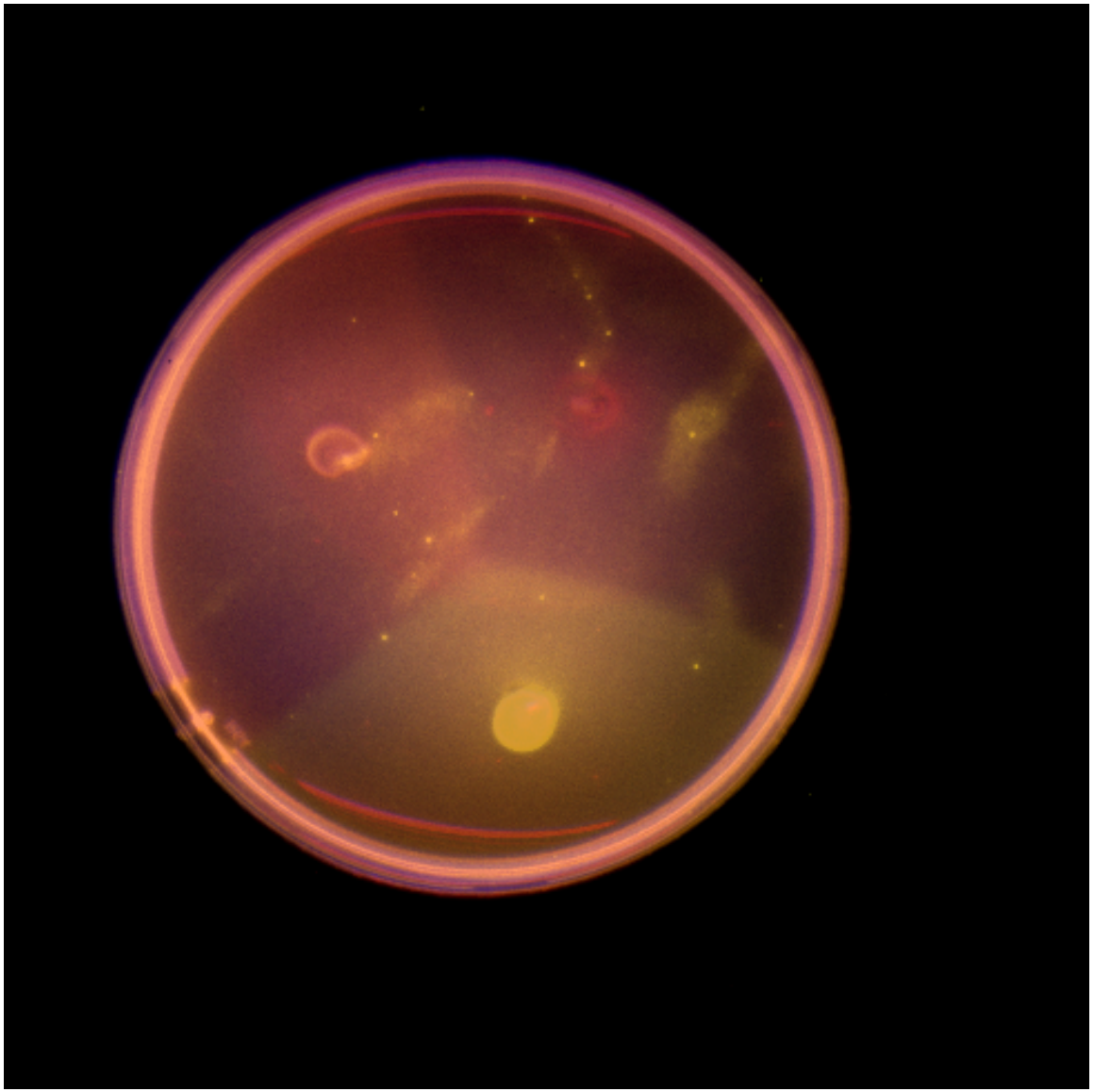


Figure 5.1. Diagram of genetic circuitry of the proposed bi-stable switch system

•So the type of Fluorescent protein expressed depends on numbers/ concentrations of types.



Uses in our project?

- Switching from recruiting to pushing
- Over complicated way?
- Reversibility?
- All biobricks already available,
- Proposed variations, using different HSL systems

- <http://www.ccbi.cam.ac.uk/iGEM2006/index.php/Main_Page>

Experimental Protocols

Rice 2006/2007– Seek and destroy E.coli

- Produced a chimeric LuxN-tsr receptor which proved functional in swarm assays - but not applicable to our project

- Swarm assay
  - Used plates of Tryptone soft agar (TSA), and made 13 equidistant spots of chemoattractant down the midline of the plate.
  - Chemoattractant gradient extends from the midline in Gaussian distribution – Derr P, Boder E, Goulian M. (2006) Changing the specificity of a bacterial chemoreceptor. *J. Mol. Biol*. **355**(5):923-32.
  - Transformed cells spotted onto TSA plates and grown at 30°C. Imgaes taken at different time points.