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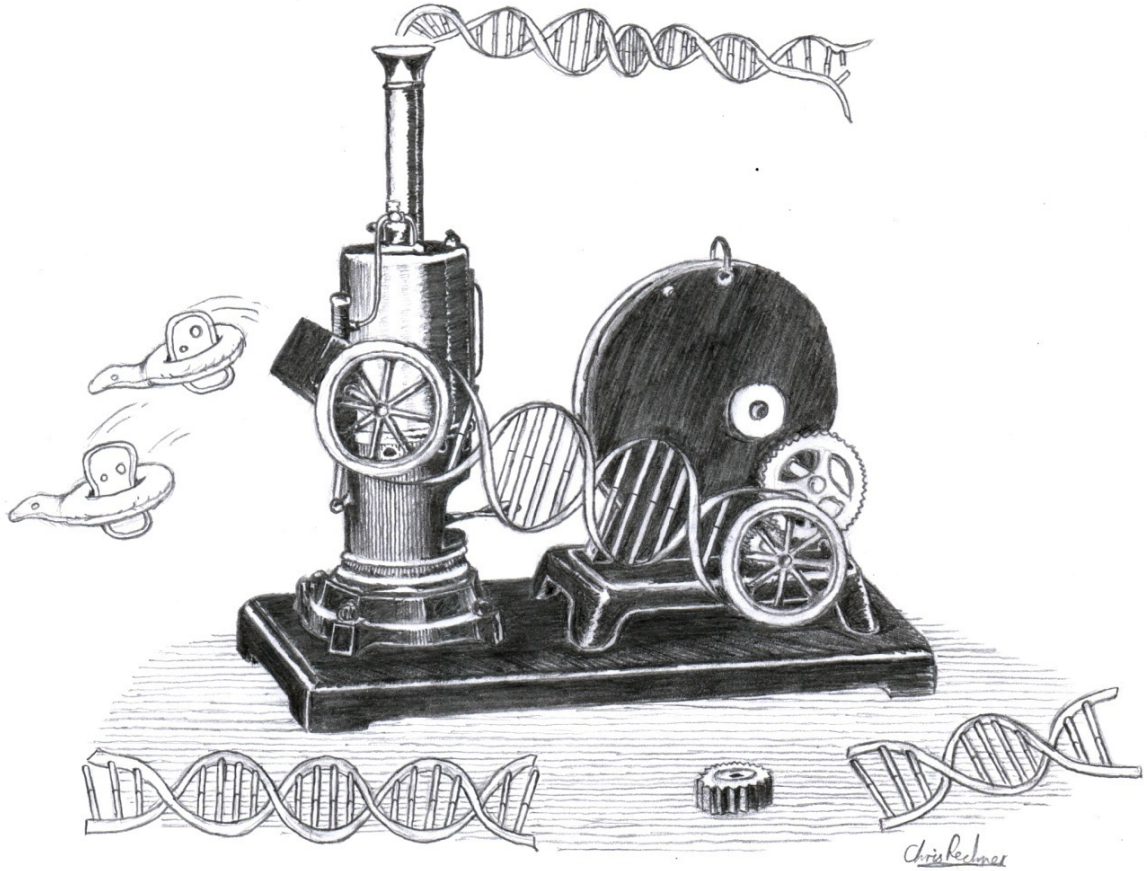
bio21 institute



iGEM Melbourne 2007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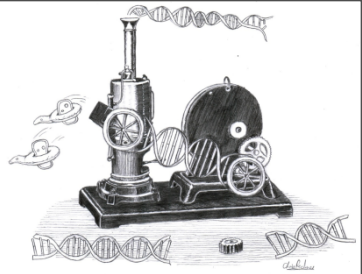
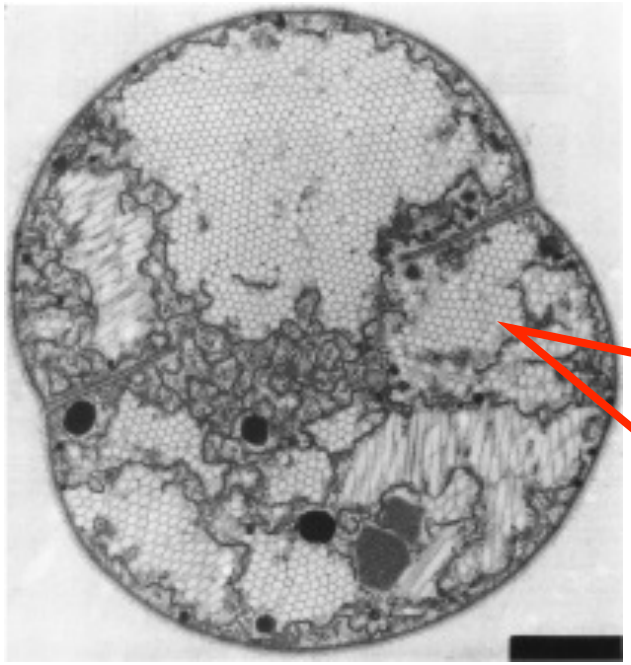
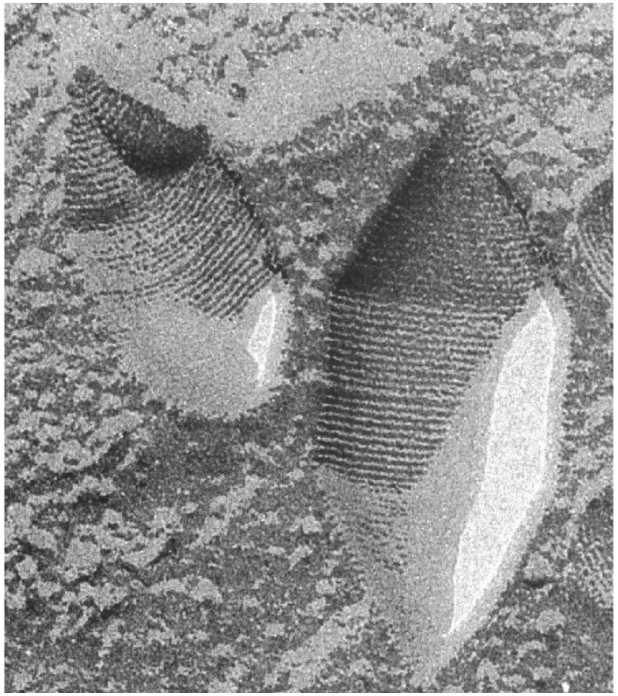
Lei's Vision

Floatation Device

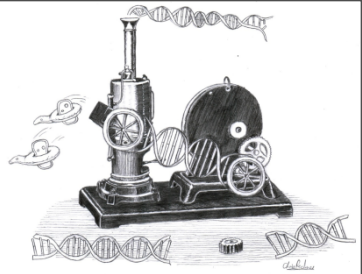


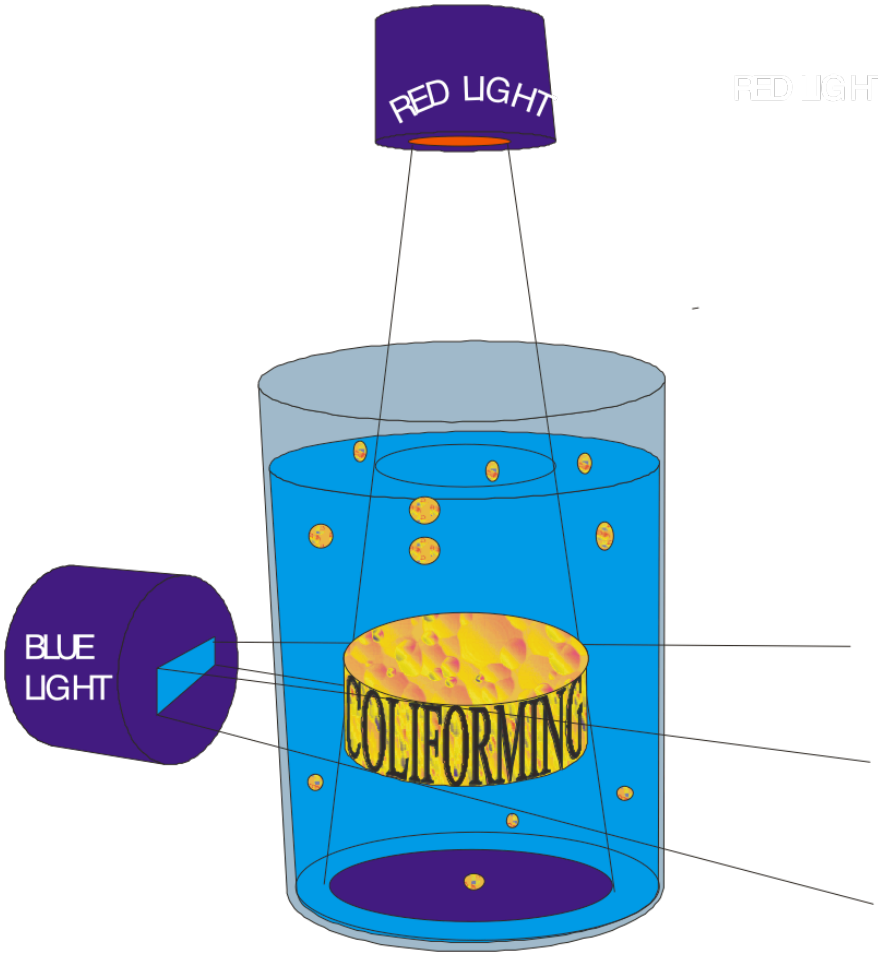




Walsby, A.E., *GAS VESICLES.* Microbiological Reviews, 1994. **58**(1): p. 94-144.

Putting it together

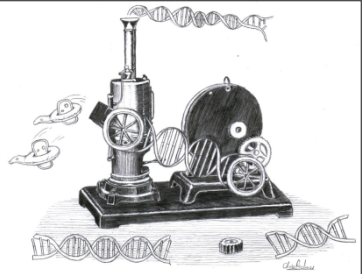




Components required:

1. **Buoyant Bacteria**
2. **Red Photosensor**
3. **Blue Photosensor**
4. **AND gate**
5. **Adhesion**

Back of the envelope…



Stoke's Law, $f = 6\pi\mu r$

Radius of bacteria = 0.5μm

viscosity of media = 1×10^{-3} Pa.s

Then, Stokes-Einstein Relationship $D = \dfrac{k_B T}{f}$

Boltzmann's constant

Temperature (K)

Frictional force

From Brownian motion: 3D random walk $t = \dfrac{\left\langle \vec{R}(t)^2 \right\rangle}{6D}$

Mean squared Displacement

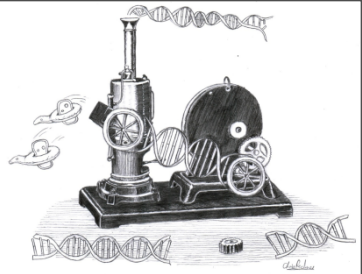
Time

Diffusivity

Therefore, for 1 bacteria to move 1mm takes 4.5 days

… and for a clump 10 bacteria across to move 1mm takes 45 days

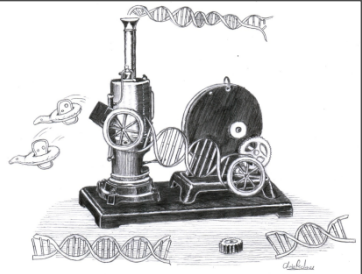
… and on the front

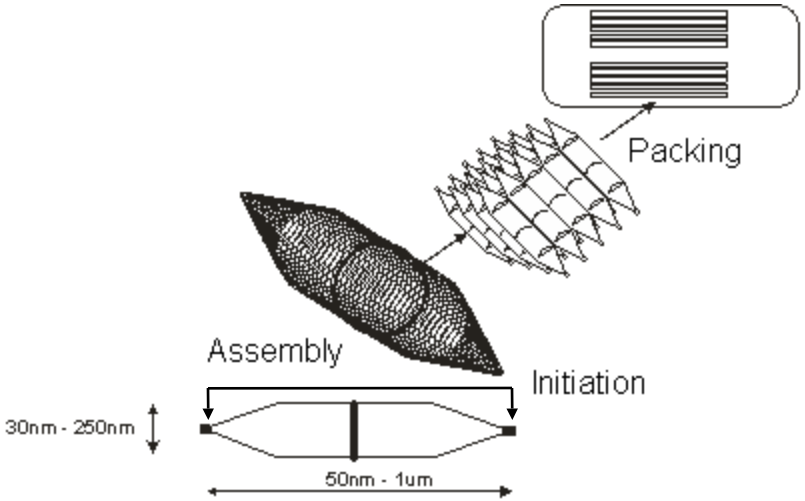


If there is enough adhesion, bacteria should effectively not move within the time scale of our experiments

Floatation

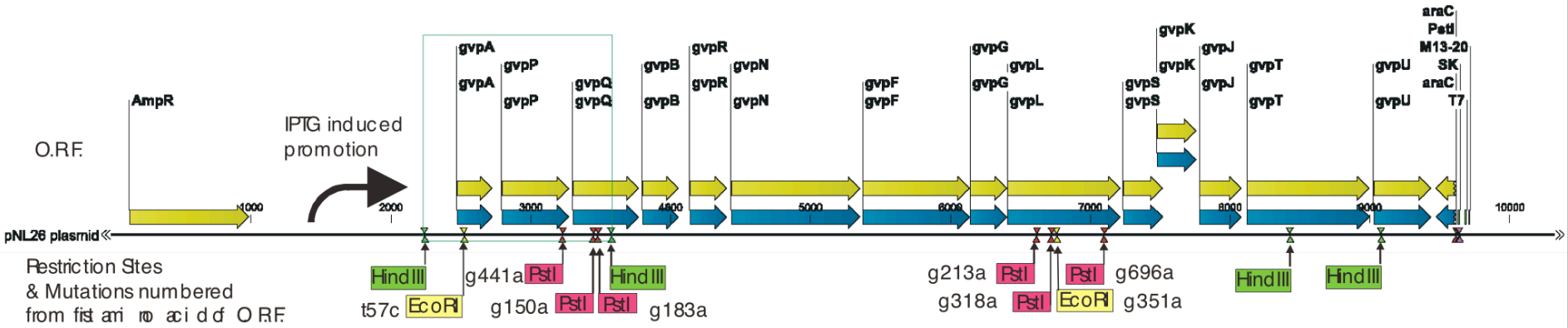
Floatation device: Gas Vesicles





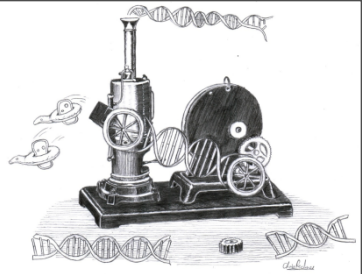
- From *Bacillus megaterium*

- Protein polymer creates a coat that embodies air, making bacteria float.
- Floatation function encoded by a gene cluster 6kb in length.
- Gene cluster can function as a polycistron.



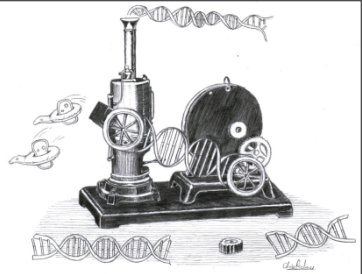
Protein	AA Length	Proposed Function
gvpB	88	Main structural protein
gvpF	255	Nucleation
gvpG	88	Present in immunoblots of vesicles
gypJ	100	Change in coil size with development
gvpK	94	
gypL	269	Coiled coil nucleation
gvpN	308	Vesicle number regulation
gvpQ	157	Negative regulation of vesicle production
gvpR	88	
gvpS	95	Similar to gvpB and gvpJ
gvpT	292	
gvpU	138	

GvP Biobrick: Plan

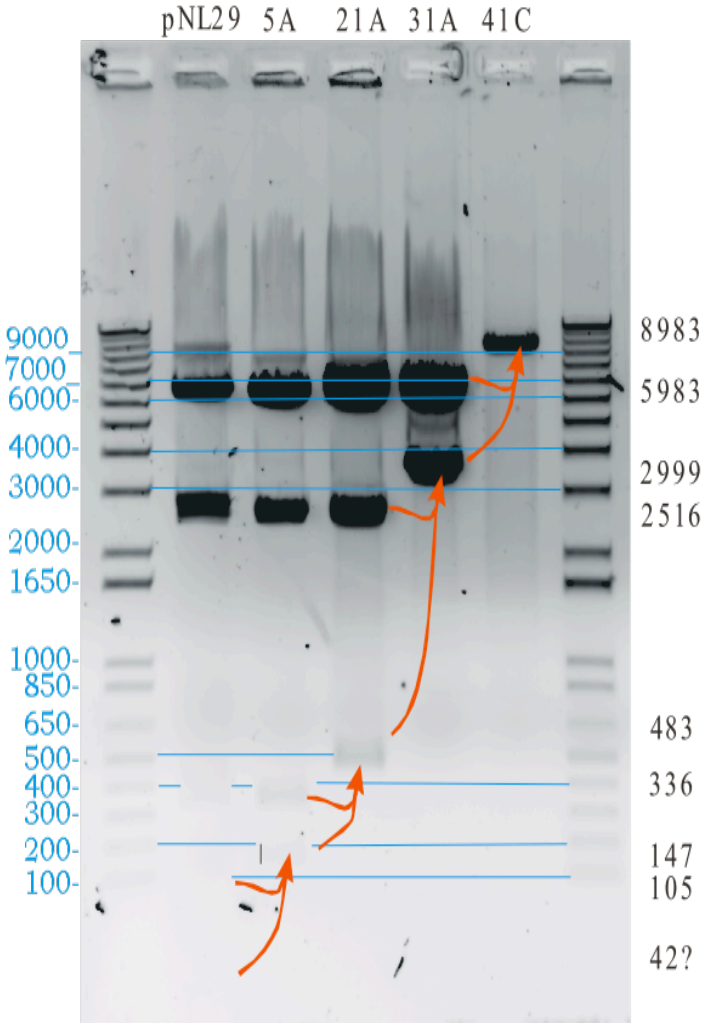


- Remove restriction sites
- Make biobricks out of all genes
- add prefix and suffix

Results



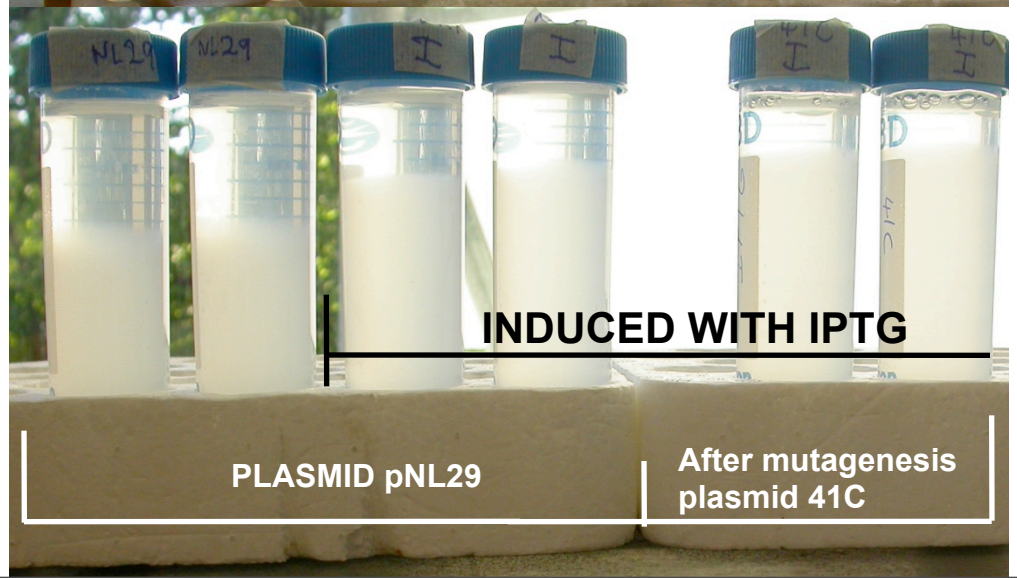
Site directed mutagenesis
EcoRI and PstI digests at each step.



- Four unintended (silent) mutations in 6kb
- 11x40bp repeat in GvPL, but doesn't seem to affect buoyancy
- BBa_I750016

Results

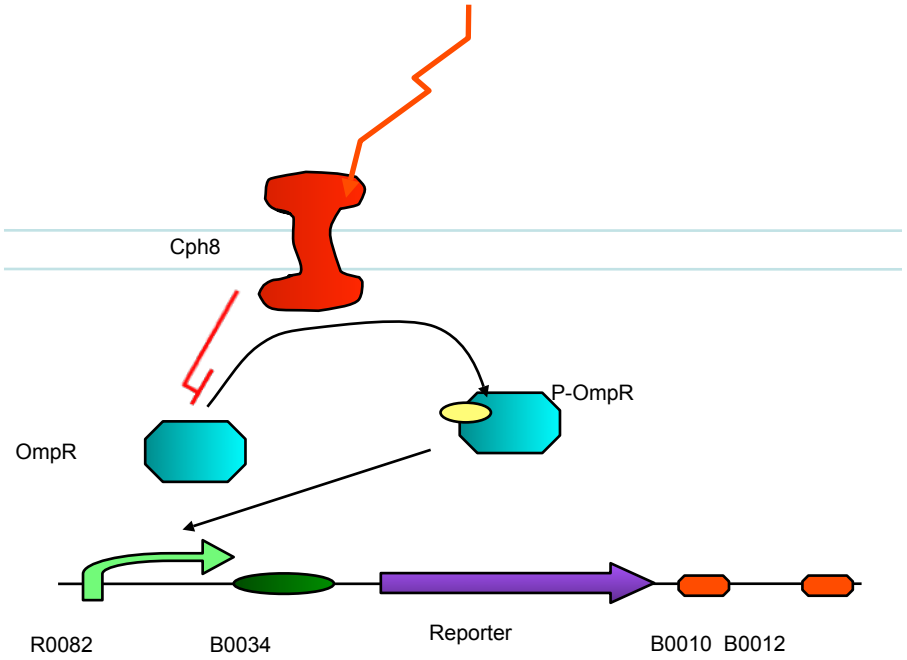
Day 0



Day 3
(72 hours)

Red Light

Red Light receptor

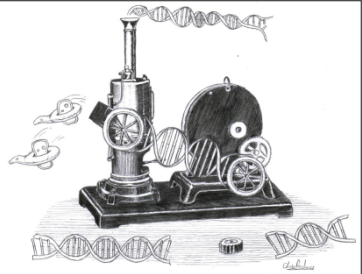


We attempted to use the Cph8 Red light receptor from the registry. (BBa_M30109)

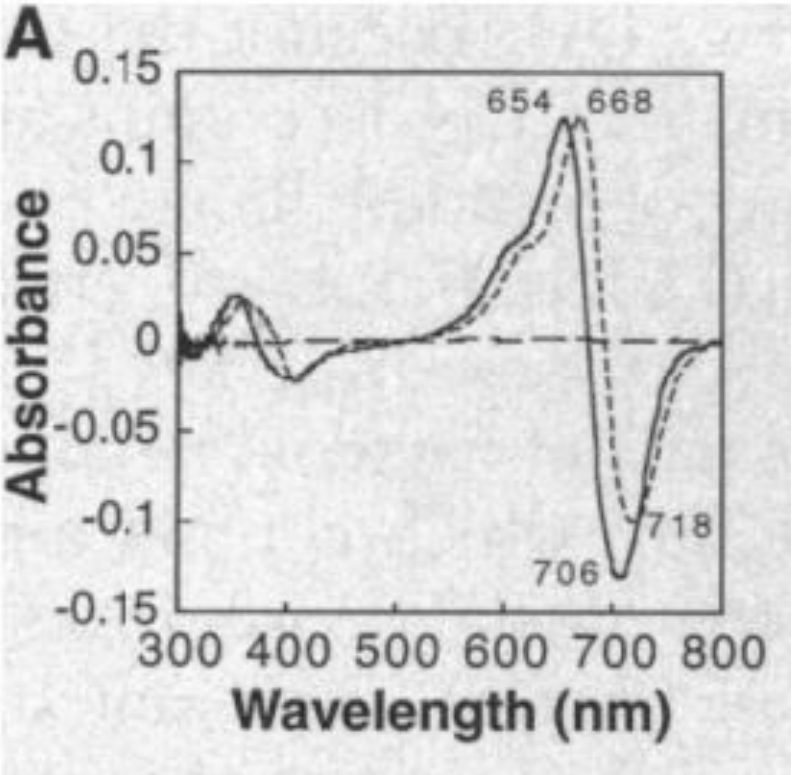
Requires PcyA and ho1

Blue Light

Blue Light Receptor

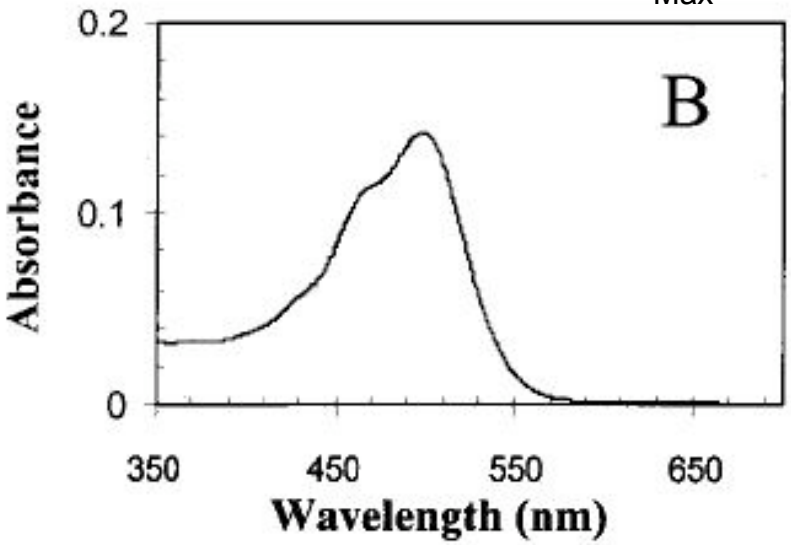


Absorbance for Cph1 A_{Max}=660nm



Absorbance for SopII

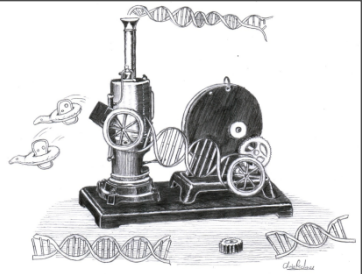
A_{Max}= 500nm



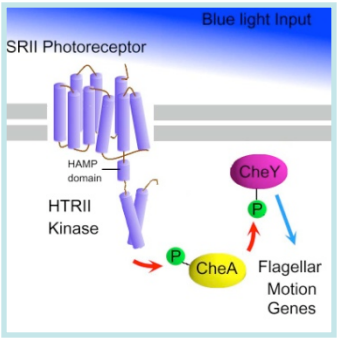
Kuo-Chen Yeh, Shu-Hsing Wu, John T. Murphy,(1997) **A Cyanobacterial Phytochrome Two-Component Light Sensory System,** *Science, 277: 1505-1508.*

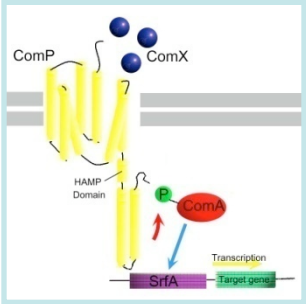
Kwang-hwan Jung, Elena N. Spudich, Vishwa D. Trivedi, John L. Spudich (2001) **An Archaeal Photosignal-Transducing Module Mediates Phototaxis in *Escherichia coli,*** J. Bacteriol. 983(21):6365–6371

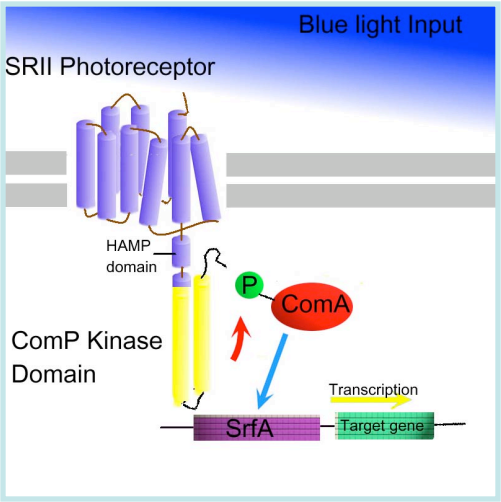
Blue Light Photosensor



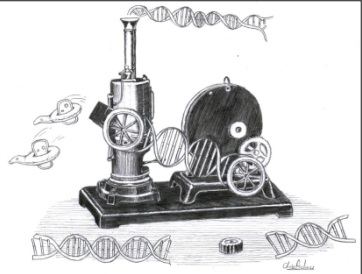
As was done with Cph8, we made a Photoreceptor-kinase chimera

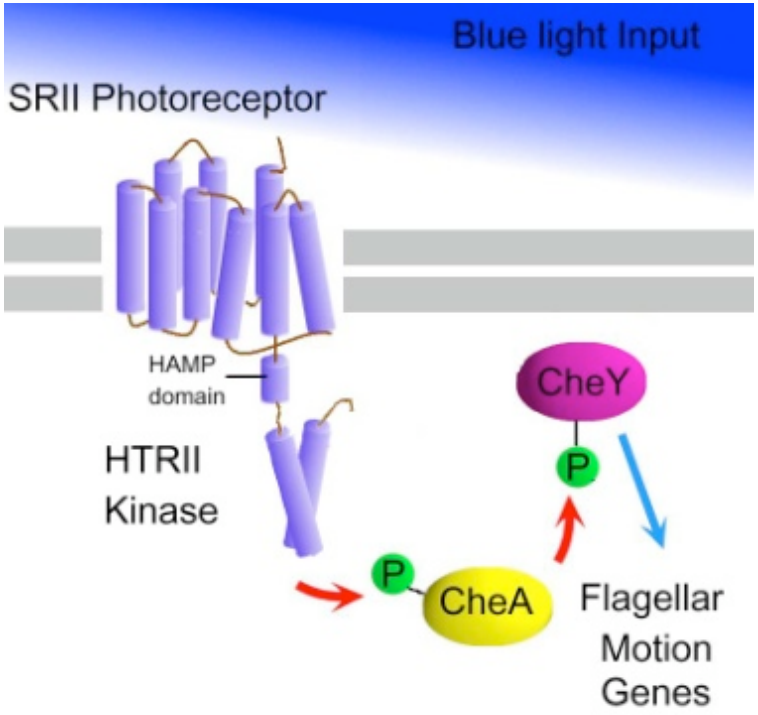






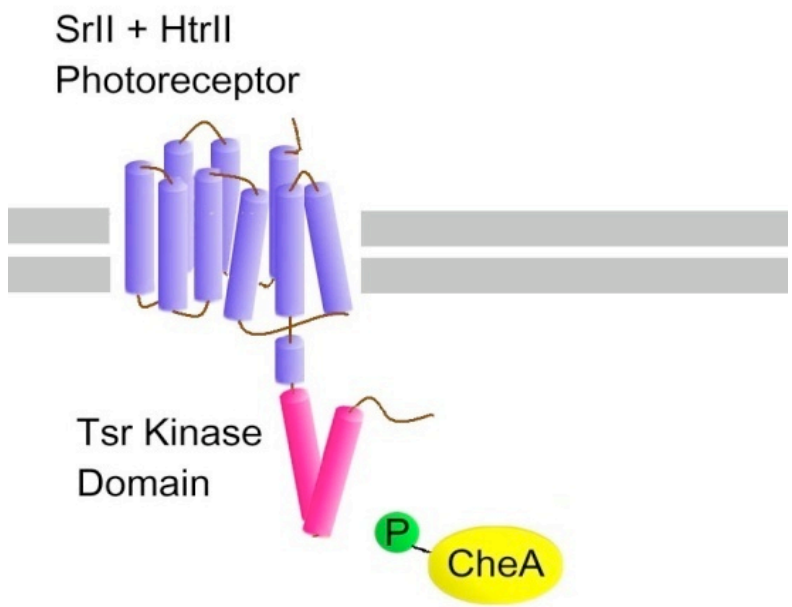
SopII-HtrII





Transmembrane protein complex, inducing flagellar motion in response to light (phototaxis)

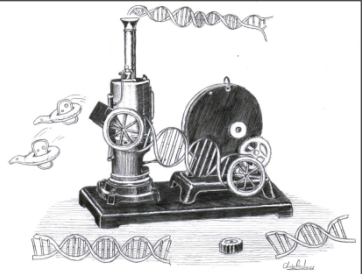
Previous work done

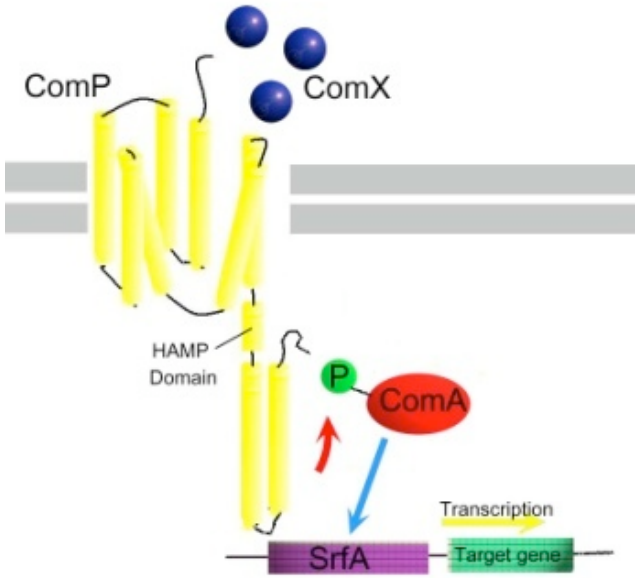


Fusion with Tsr Kinase from *E. coli* works – a two-component signal transduction is initiated in *E. coli*.

Jung *et al* (2001) *J. Bacteriology* **183** 6365-6371

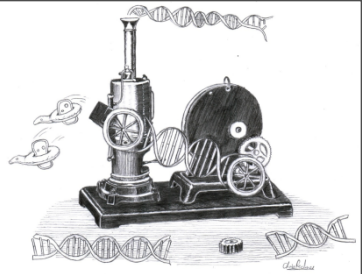
ComP/ComA

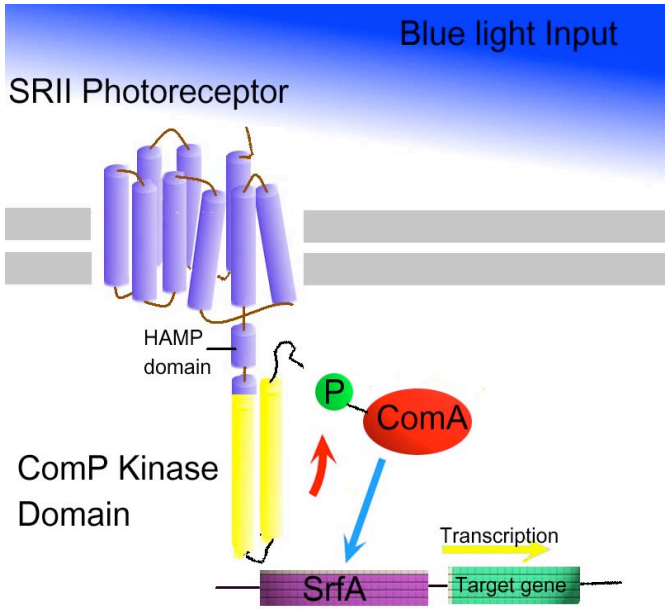




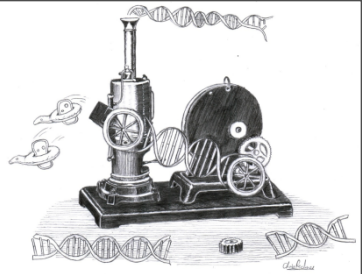
- **Exists as a part, but not in distribution**
- **Ordered from GENEART, and optimised sequences. They have have been submitted as ComPalt, ComAalt**

Selection of fusion sites

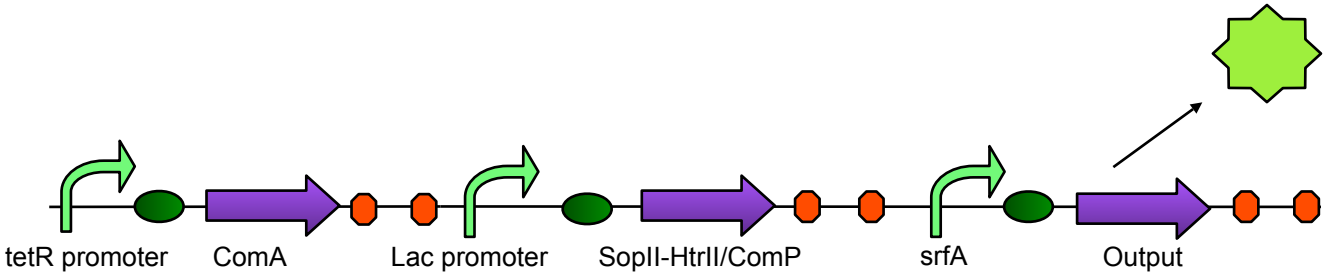




…still under construction

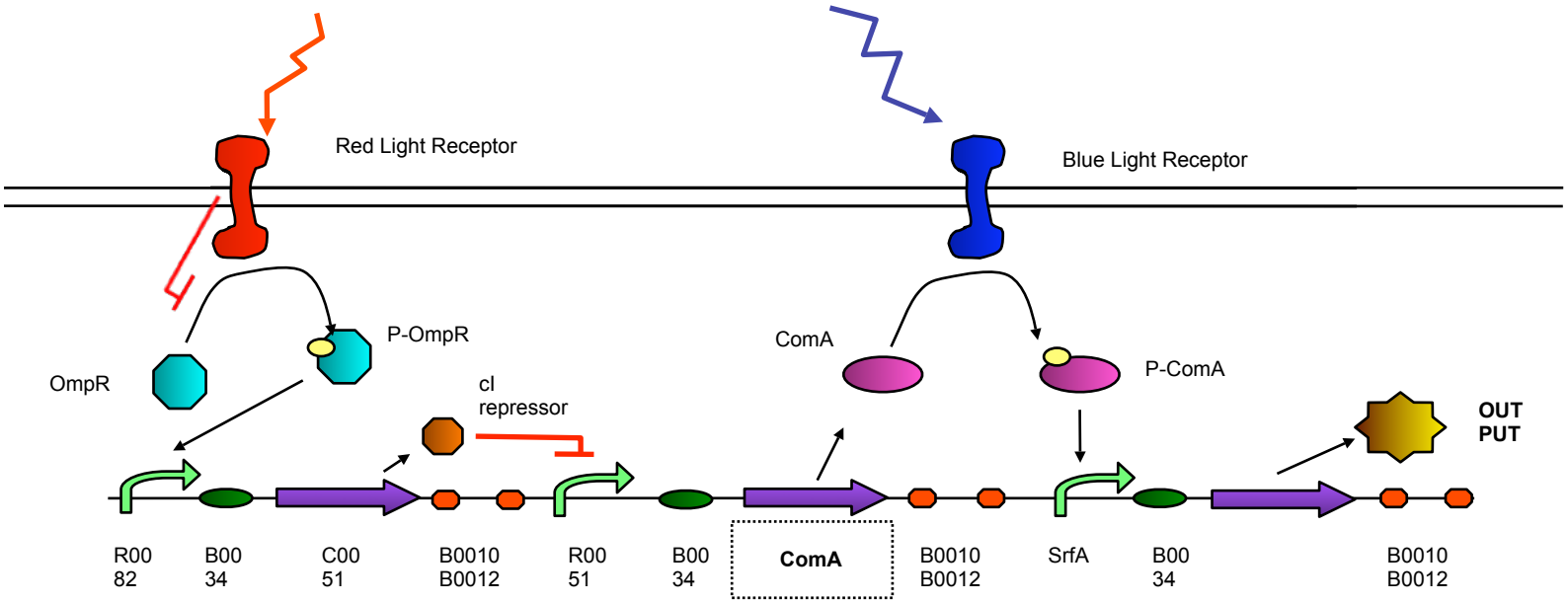


- **Fusions created by PCR stitching**
- **Have created the chimeras, but still characterizing.**

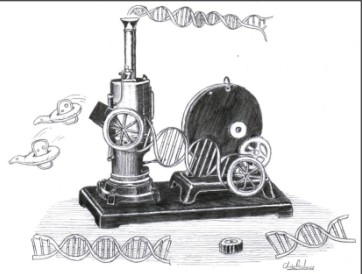


AND gate

Integration: The AND gate



Reporter Construct

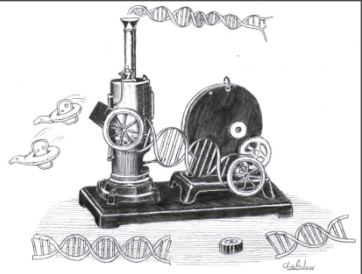


- **Currently, our construct is designed to express a reporter gene (GFP)**

- **Will show that genes under the control of srfA are up-regulated only at the intersection of the two light beams.**

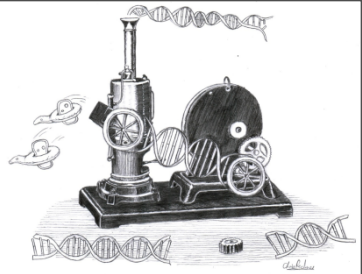
Adhesion

Possible Adhesion Systems



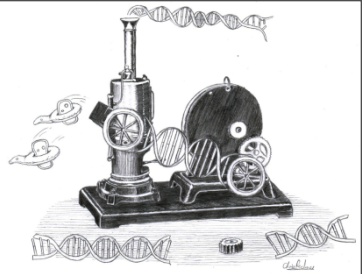
- **Surface expression of Ig-like molecules**
- **Intimin**
- **Fos-Jun adhesion molecules (McGill University)**

Medical Applications



- **Possible applications in building scaffolds in tissue engineering**
- **Cadherins**

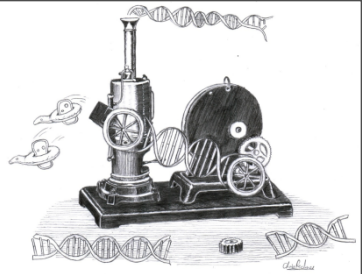
Progress…



Floatation Device is now a Biobrick

Still working on putting together the light responsive system and coupling it to the adhesion molecules.

Thank you!



- Joe (for his super-competent cells)
- Lei Xing
- Heung-Chin Cheng & Paul Gooley, and their respective labs
- University of Melbourne
  - Dept of Biomedical Engineering
  - Dept of Biochemistry and Molecular Biology
  - Bio21 Institute

  James Spudich

  Maura Cannon

  Alan Grossman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