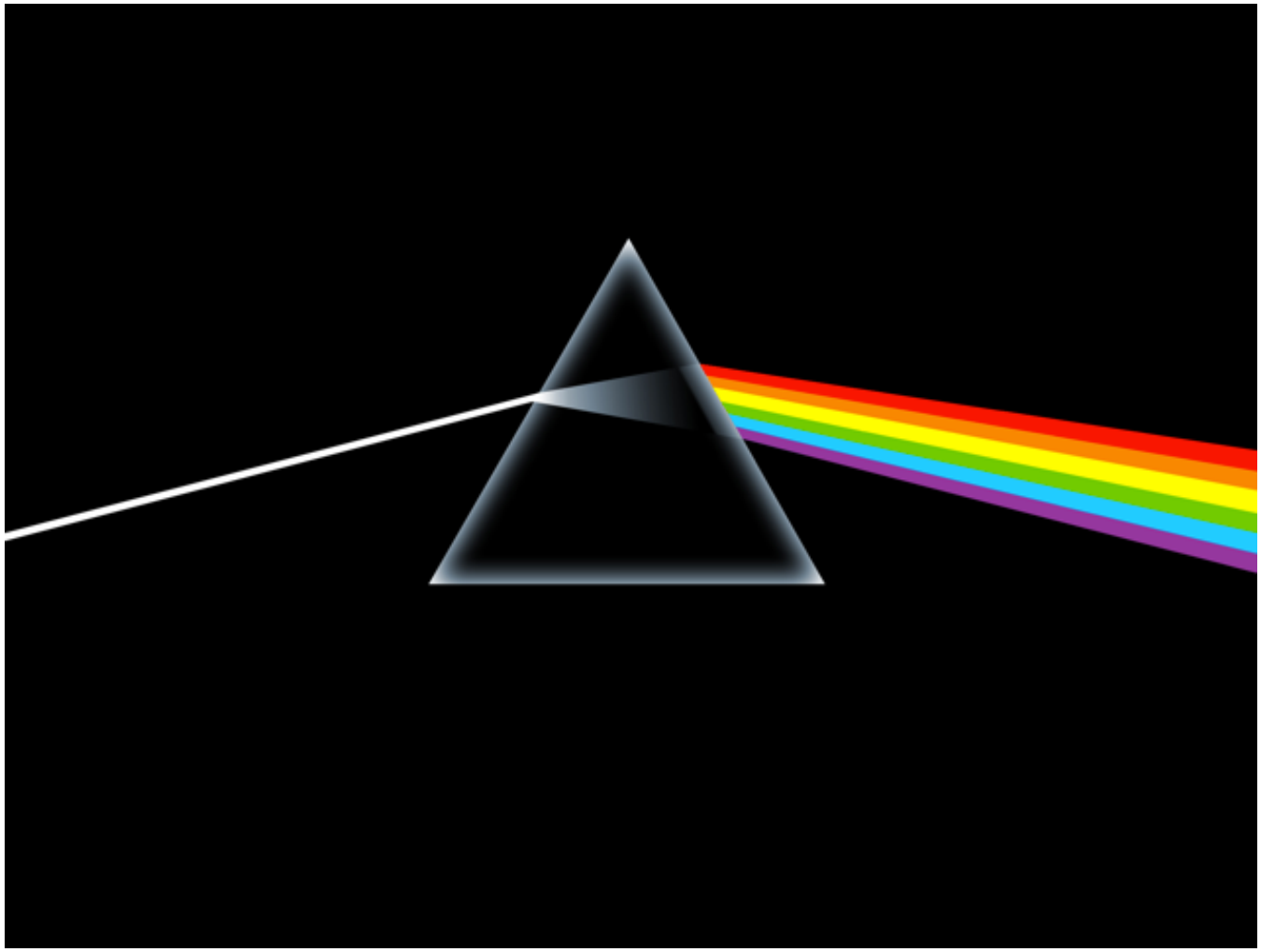
Optogenetics:
Engineering optical control of biology



Optogenetics

This lecture will cover:

- **Overview of optogenetics – why use light?**
- **Main Examples: bacterial ‘photography’ and the edge detector**
- **Brief examples of light control in other organisms: yeast and mice.**

Optical control - Overview

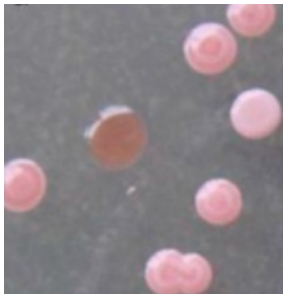
- Bacteria (and other organisms) can be engineered to ‘see’ light via expression of receptors
- Much more rapid and reversible than chemical signalling
- Can integrate light sensing in more complex circuits to program pattern formation (Elllis).
- Can interface living and electronic systems for control of gene expression and behavior

Using visible light to control biology

- Light is…
- Controllable in space, time, wavelength, etc
- Reversible
- Cheap

- There are a number of light sensors in Nature

e.g. ion pumps: halorhodopsin



Purple membrane protein in halobacterium halobium

e.g. 2 component/kinase systems: plant phytochromes

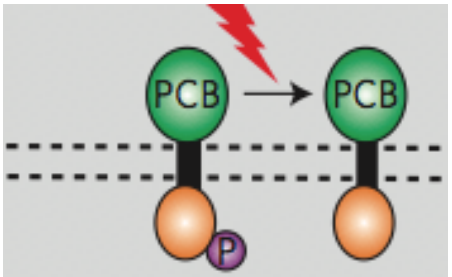
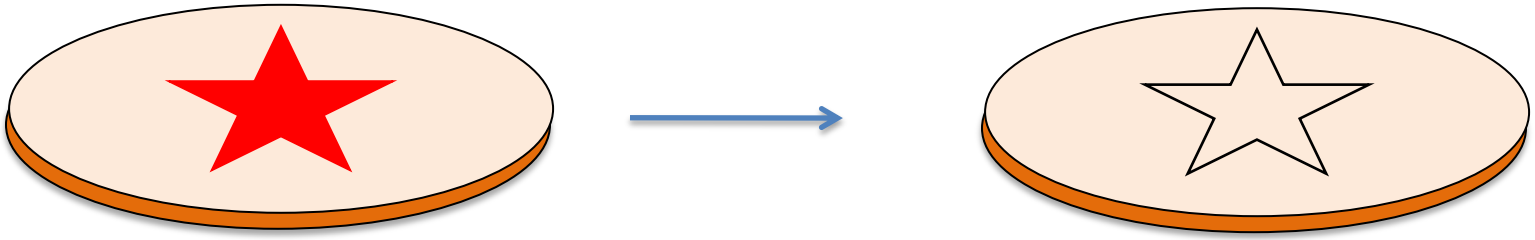


Image processing:
from bacterial ‘photography’ to the edge detector



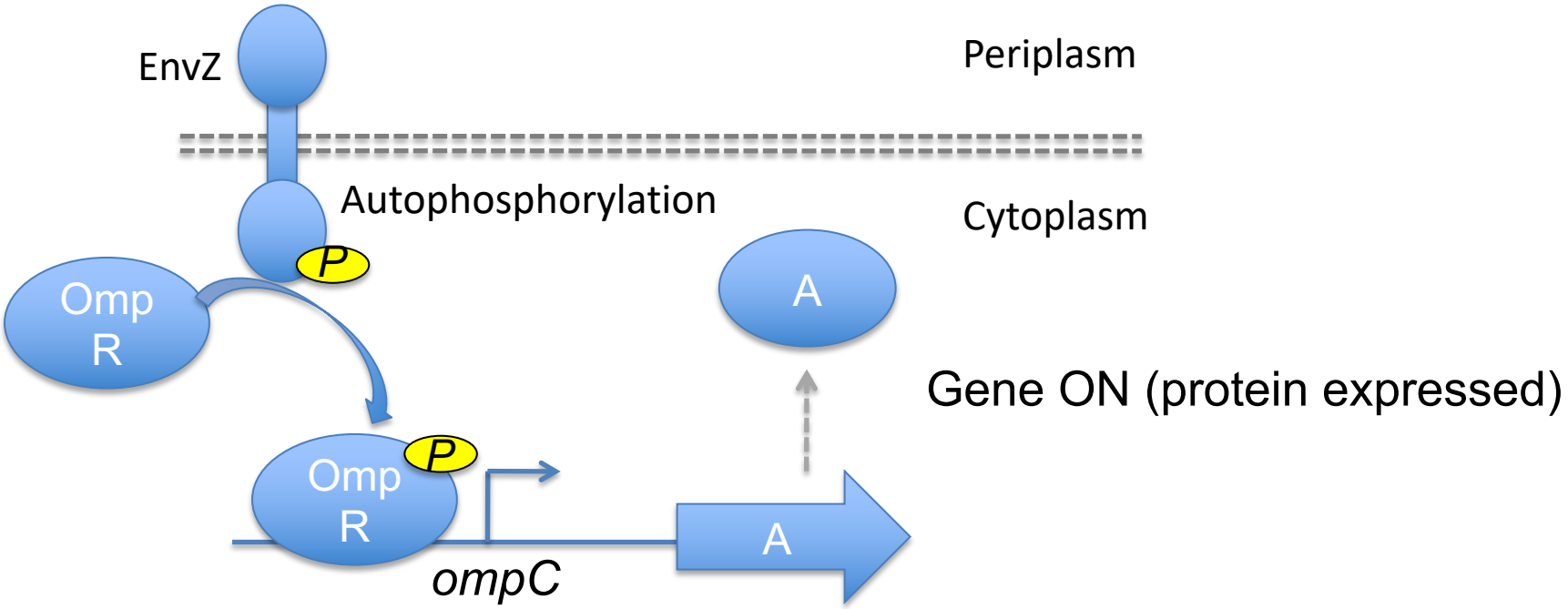
Projected image

Processed image: Edge detection

From Levskaya et al. Nature. 2005 Nov 24;438(7067):441-2.
From Tabor et al. Cell. 2009 Jun 26;137(7):1272-81.

Bacterial two component system

Bacterial two-component system



Bacterial two component system

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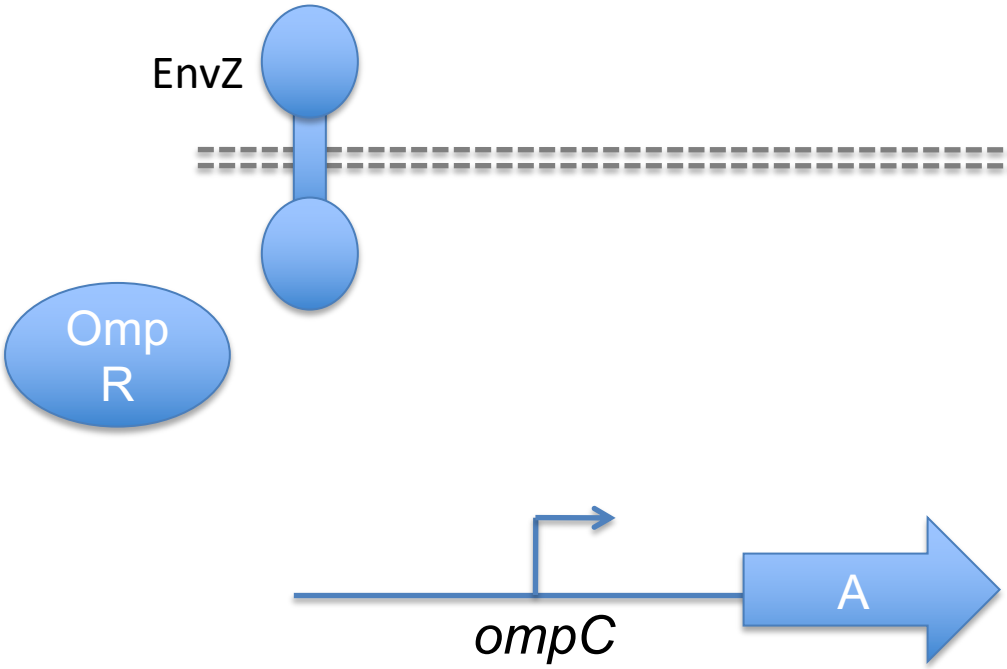
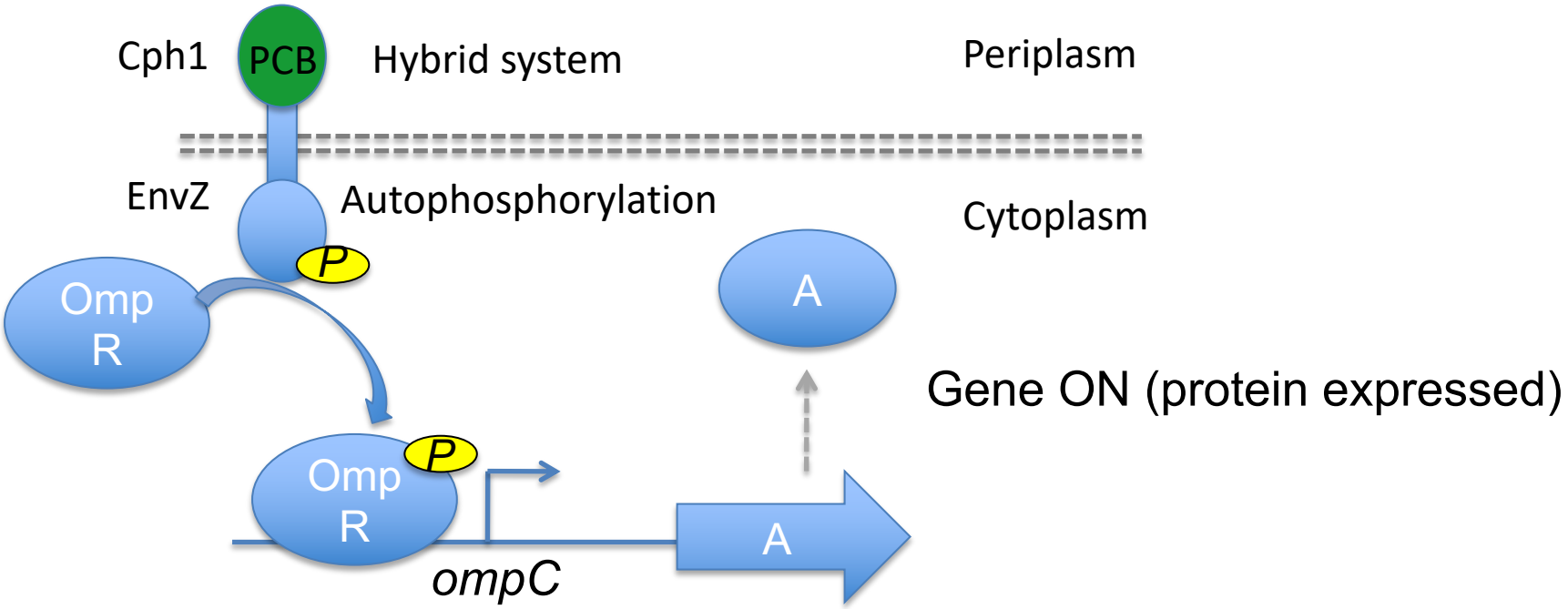


Gene OFF (no protein expressed)

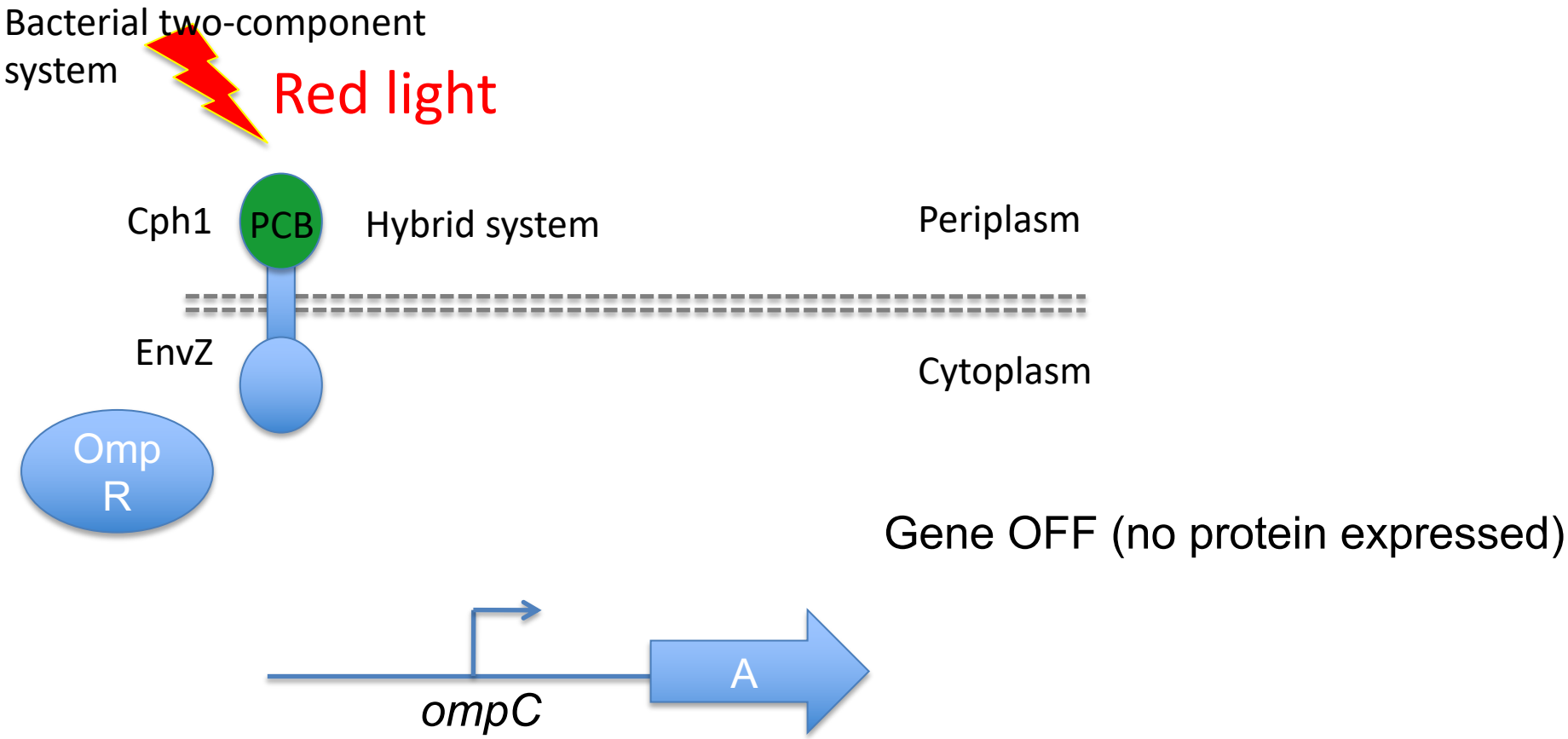
Step 1: programming bacteria to sense light

Bacterial two-component system

In dark

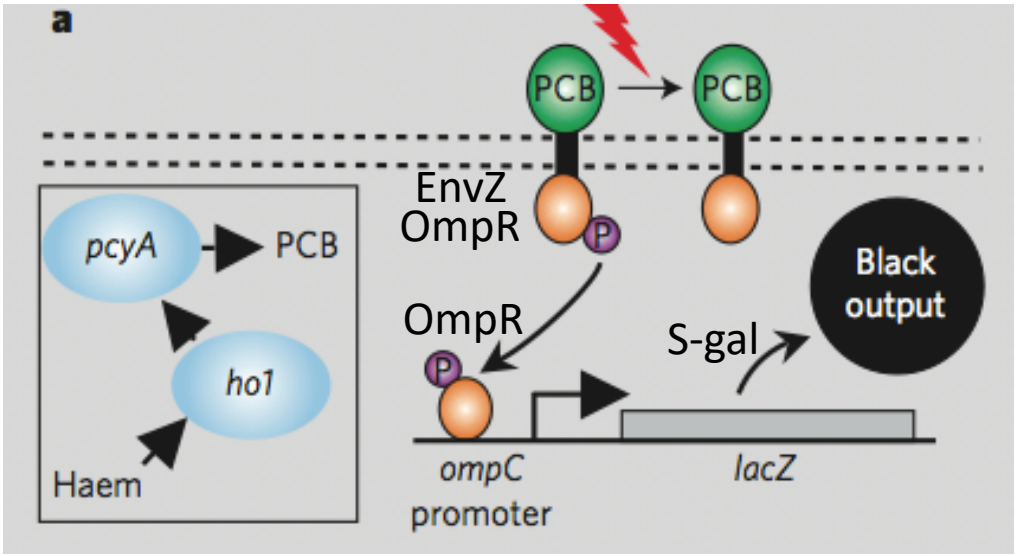


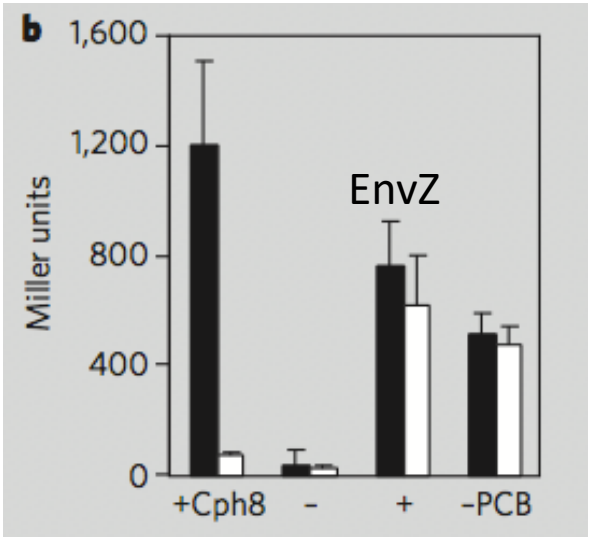
Step 1: programming bacteria to sense light



Programming bacteria to sense light

phytochrome (PCB)





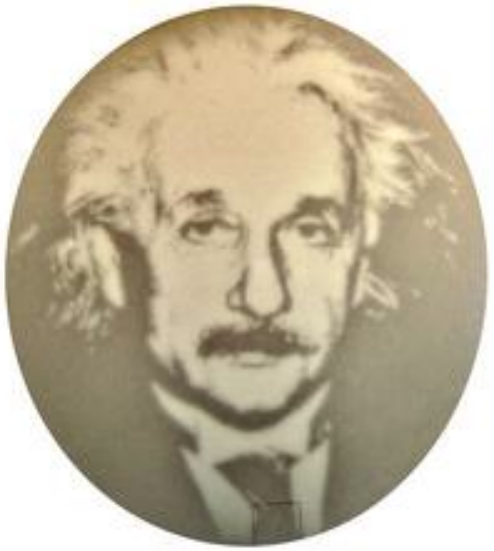
Cph8: synthetic chimaeric light receptor
Haem is converted to phycobilin (PCB) by ho1 and pcyA
PCB is the chromophore of Cph8

Programming bacteria to sense light

Projected image

Bacterial photograph

Programming bacteria to sense light



Can we design bacteria to ‘see’ and ‘process’ images?

Can we design bacteria to ‘see’ and ‘process’ images?

In biology, complex functions can be achieved by collectives of ‘simple’ agents

Genetic circuit engineering workflow

1. ‘whiteboard’ level design to achieve desired function
2. Design with actual genetics
3. Build
4. Test individual components / devices
5. Test final construct

(repeat as necessary)

The goal

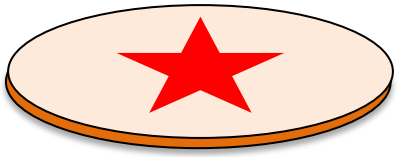
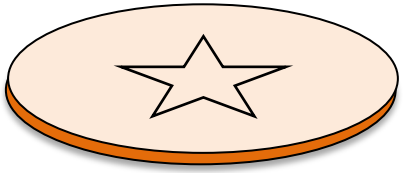
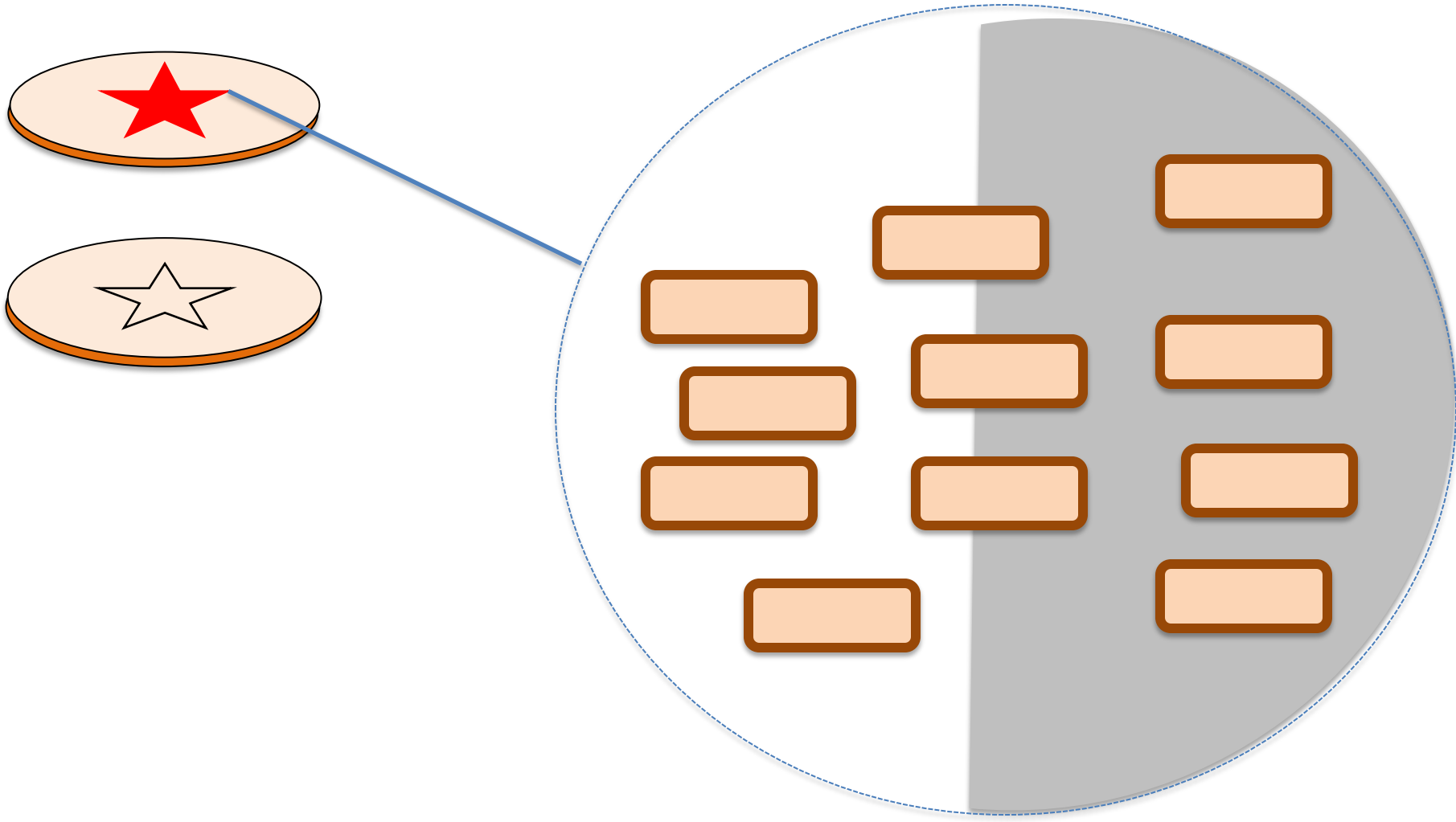


Image projected



Processed image

The logic behind edge detection



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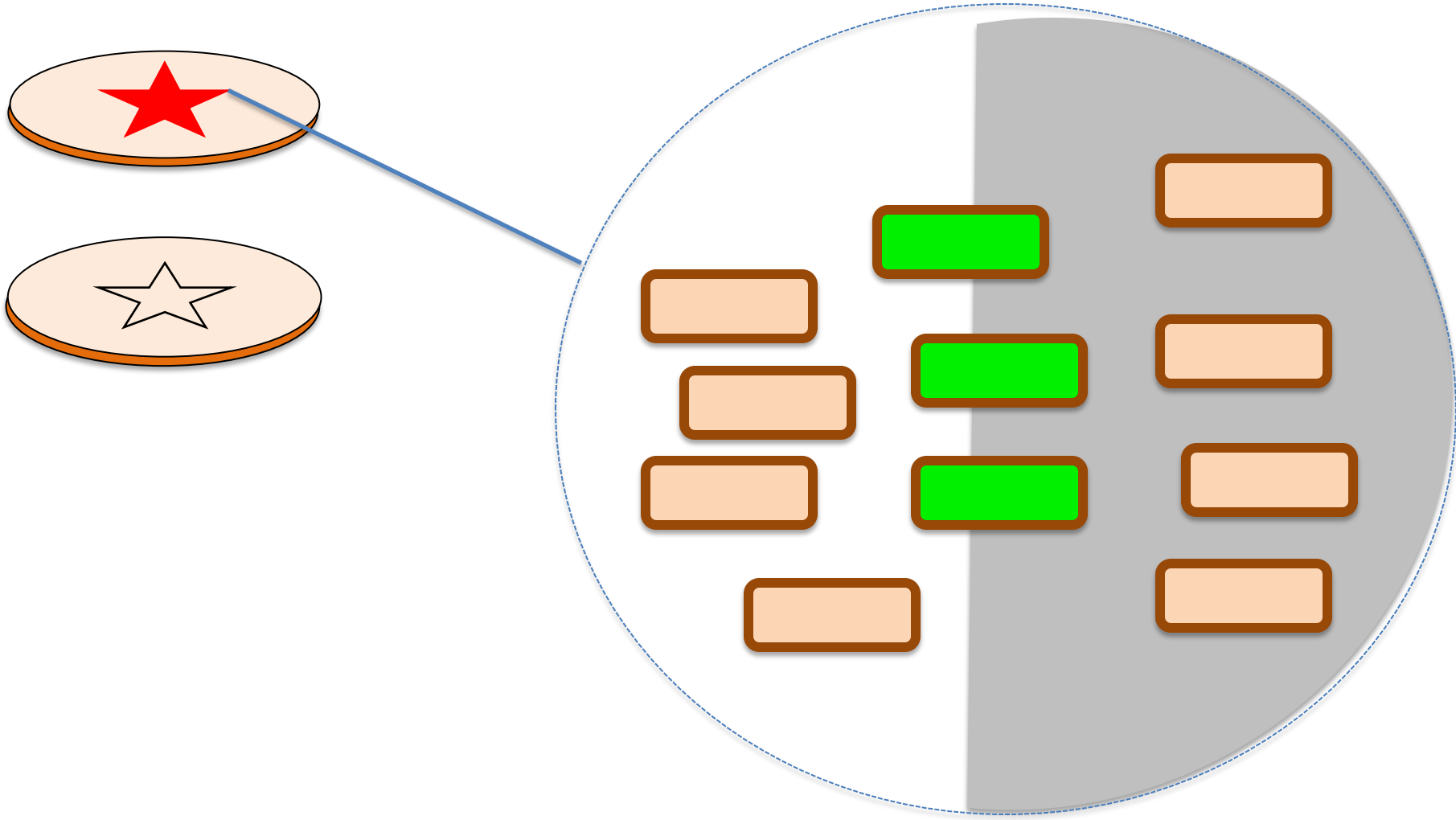

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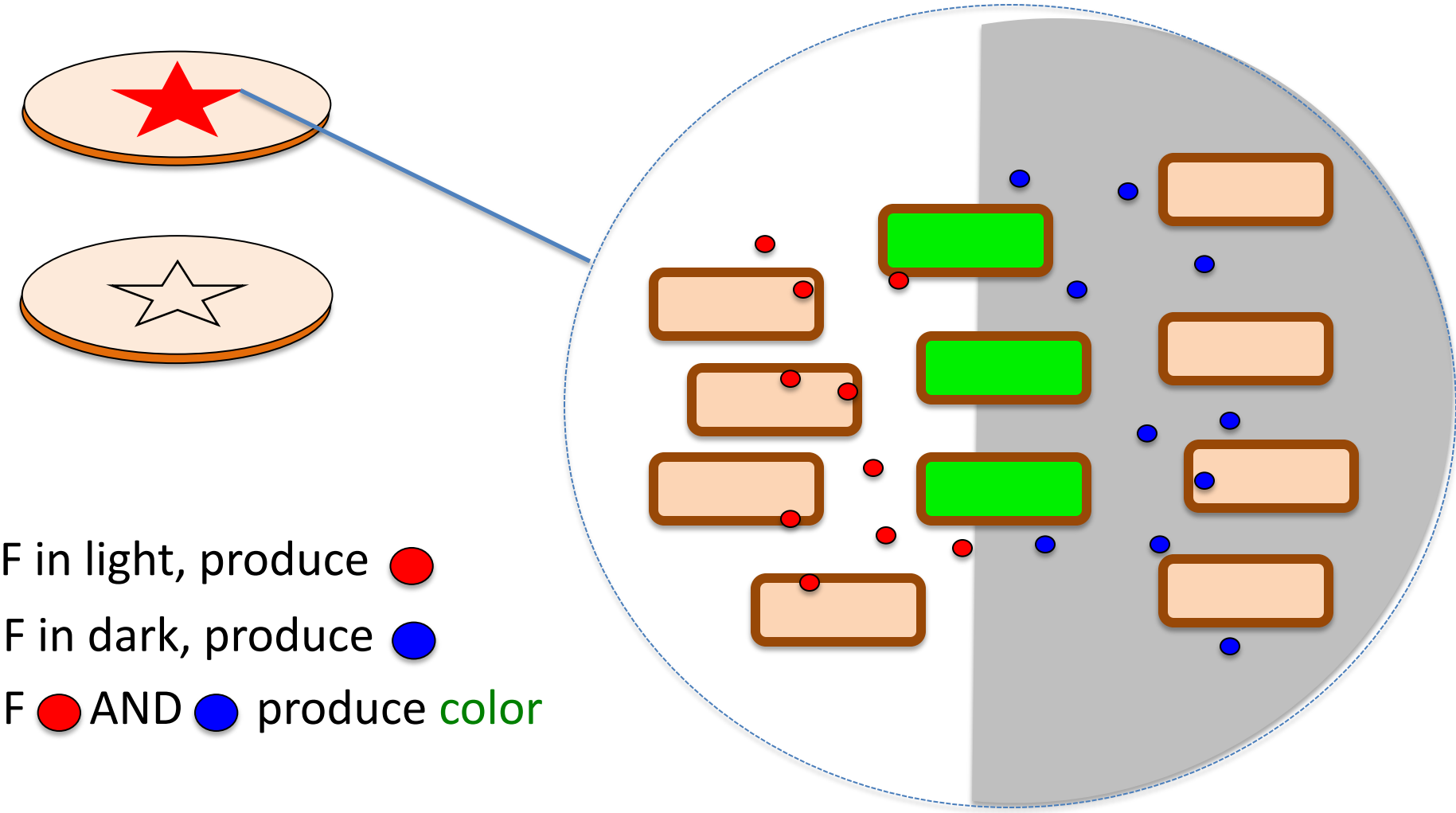
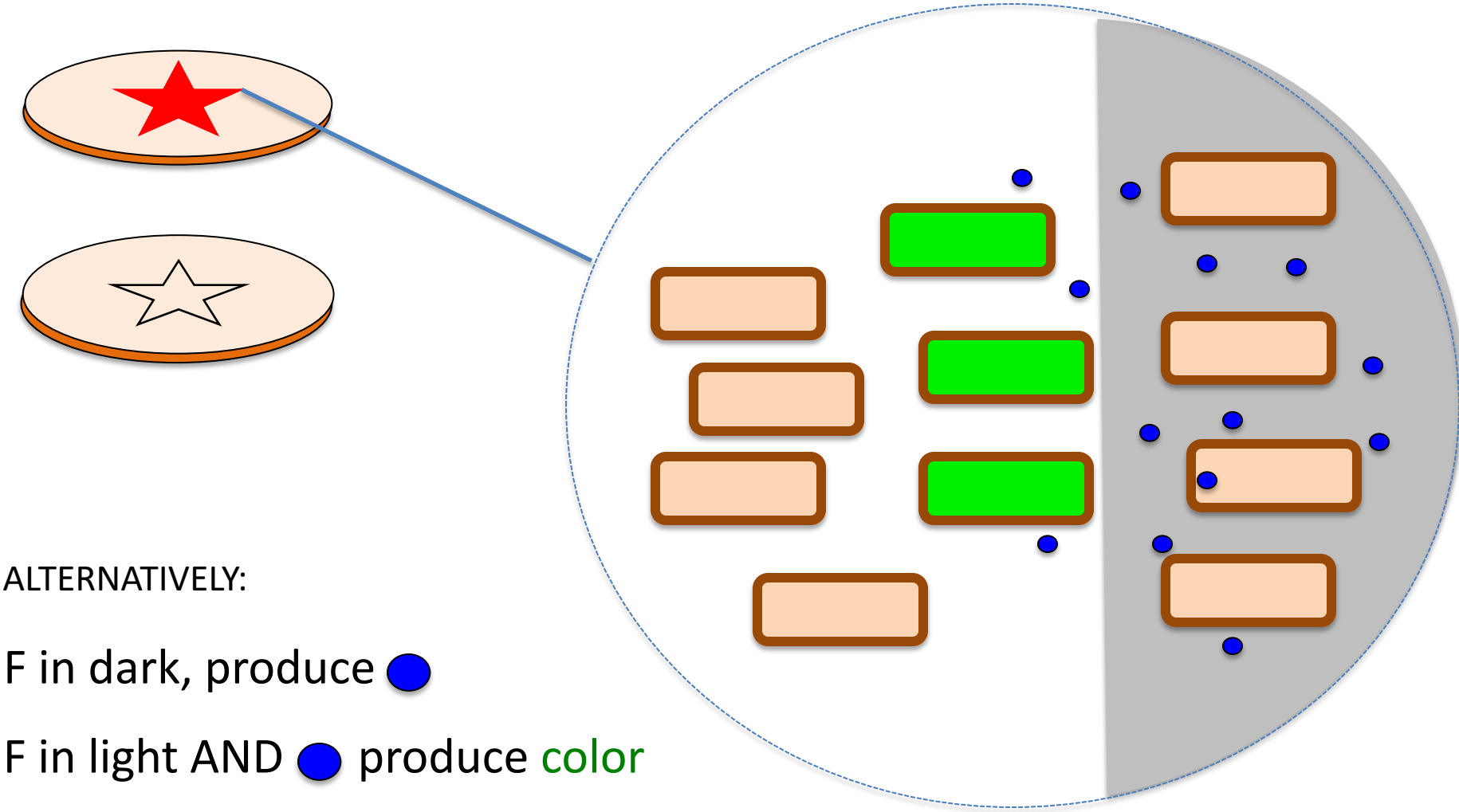


IF in light, produce

IF in dark, produce

IF AND produce color

The logic behind edge detection

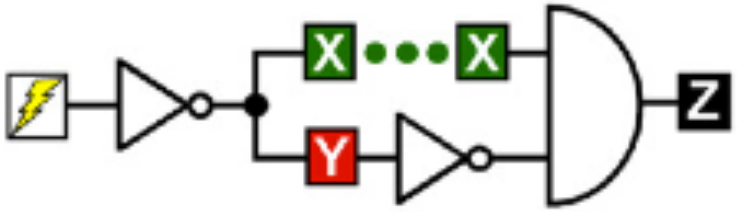


ALTERNATIVELY:

IF in dark, produce ●

IF in light AND ● produce color

From AND logic to DNA sequence



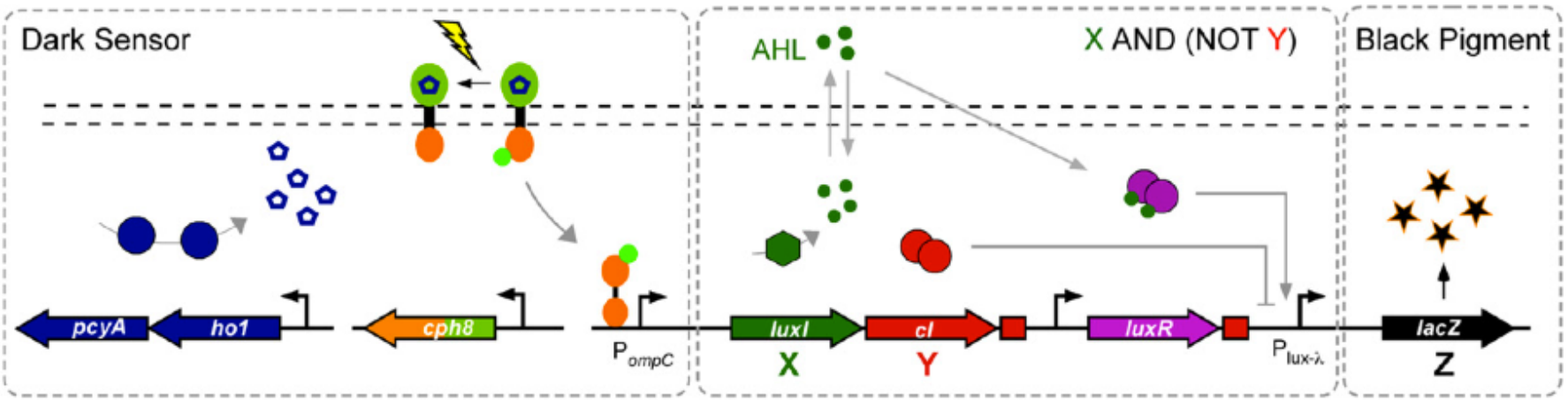
X	Y	Z
0	0	0
1	0	1
0	1	0
1	1	0

IF in dark, produce ● (AHL)

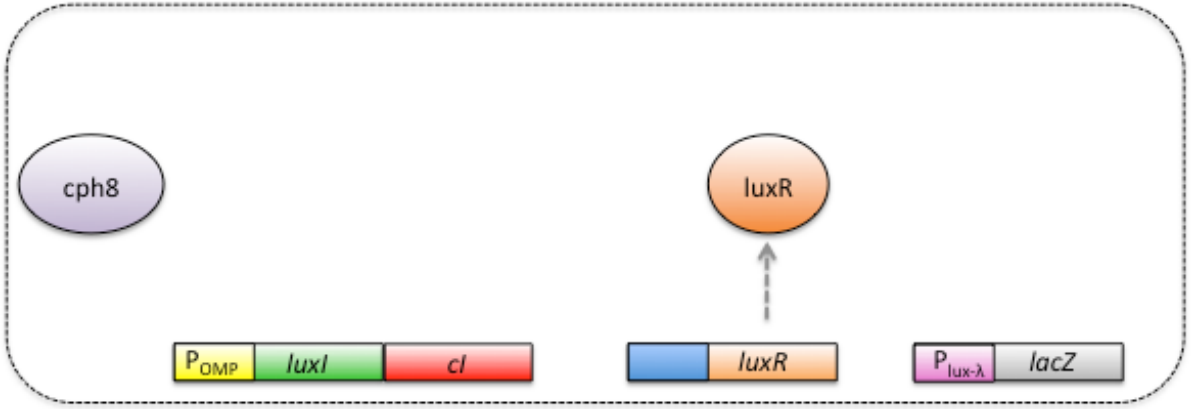
IF in light AND ● produce color

Y = Dark Sensor

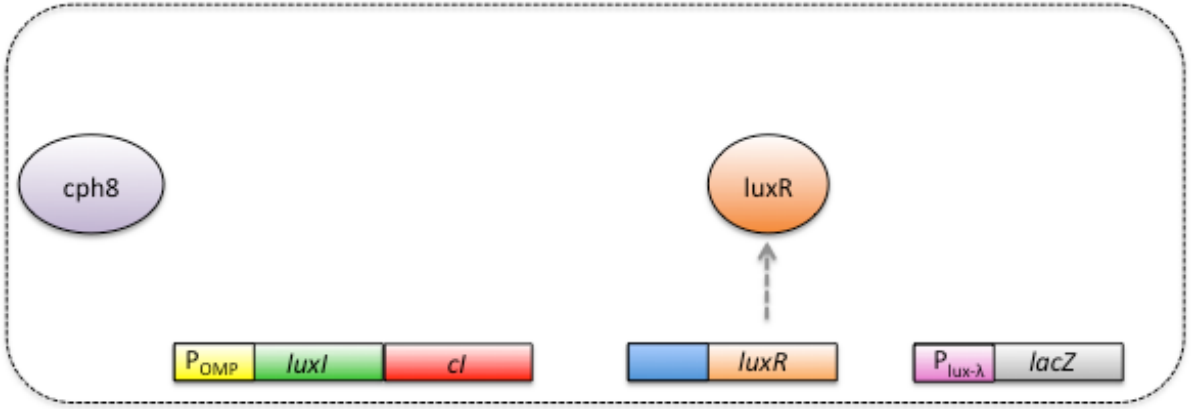
X = ● AHL sensor



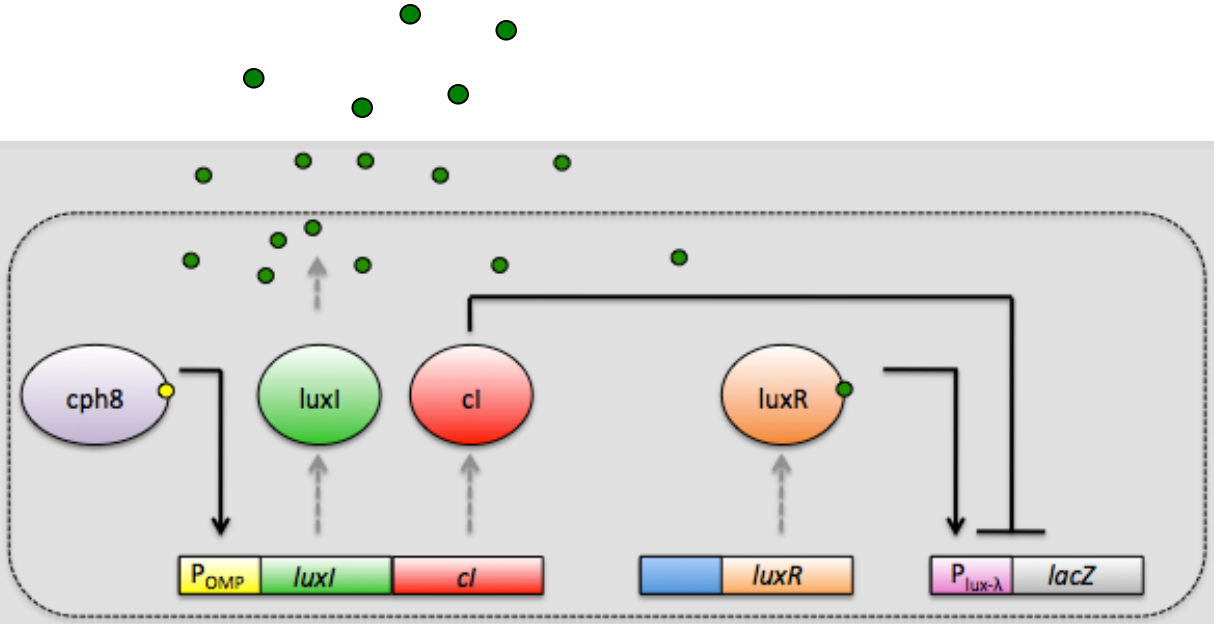
Cells in light



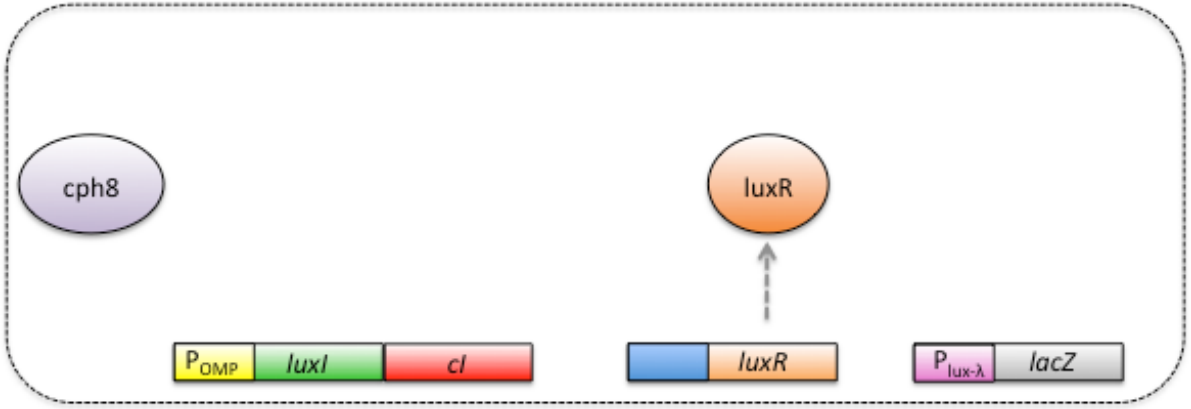
Cells in light



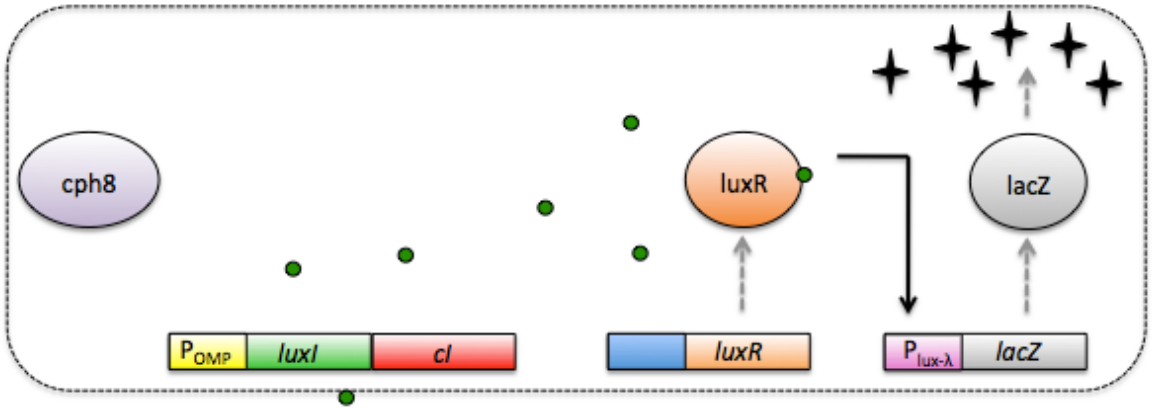
Cells in dark



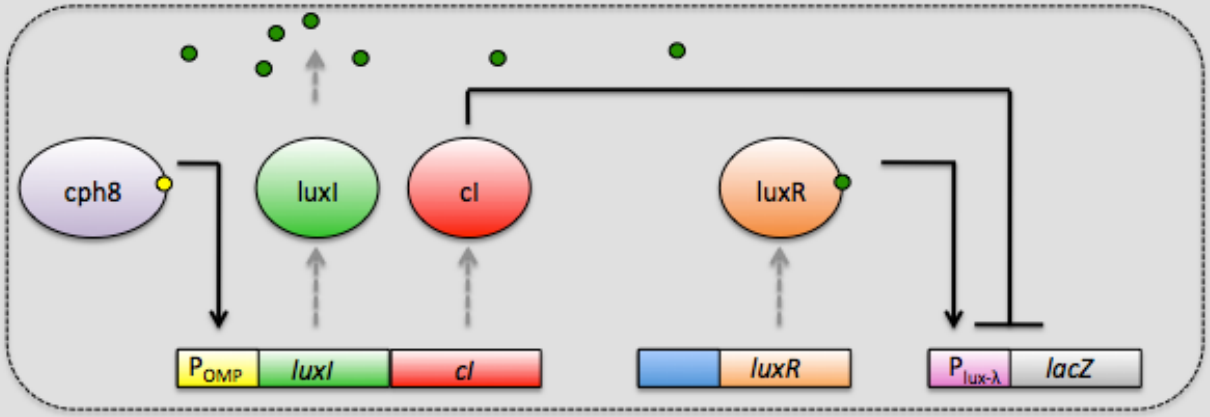
Cells in light



Cells at edge

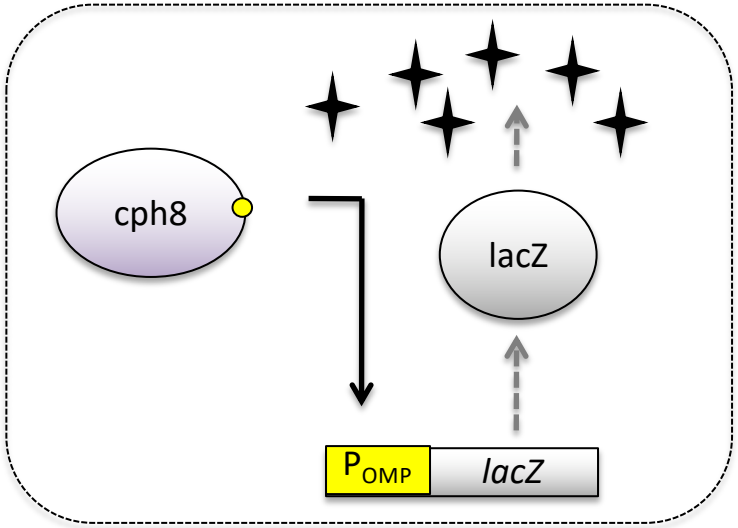


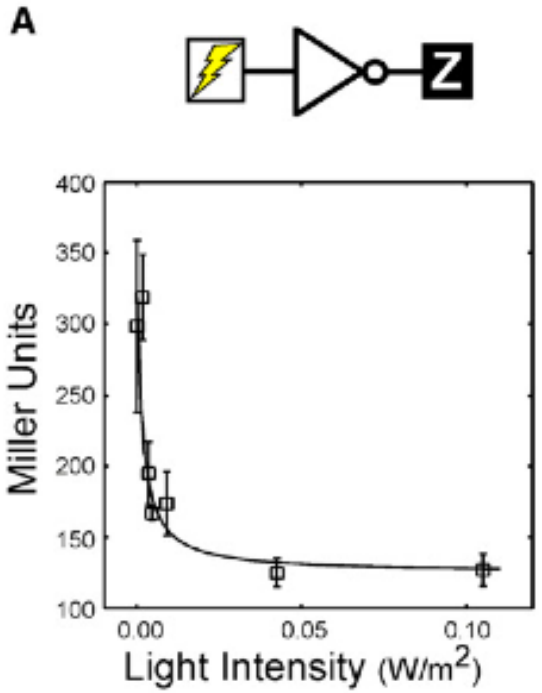
Cells in dark



Integrating genetic elements: testing individual modules

Testing photography: Dark = ON/black pigment, Light = OFF/white





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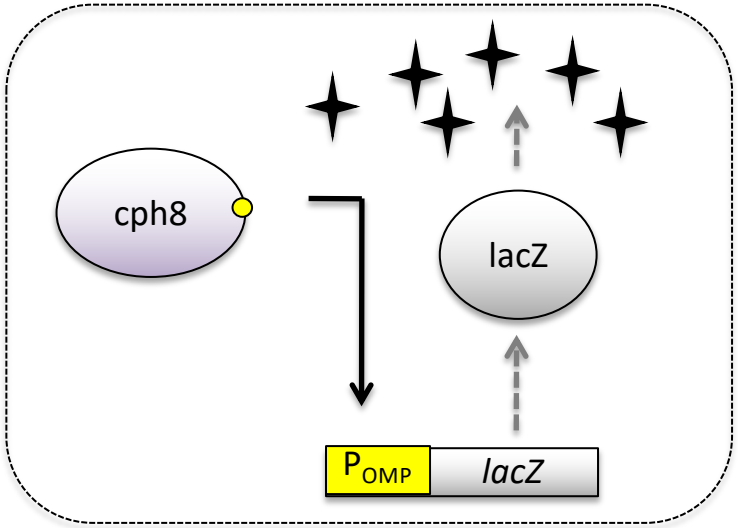
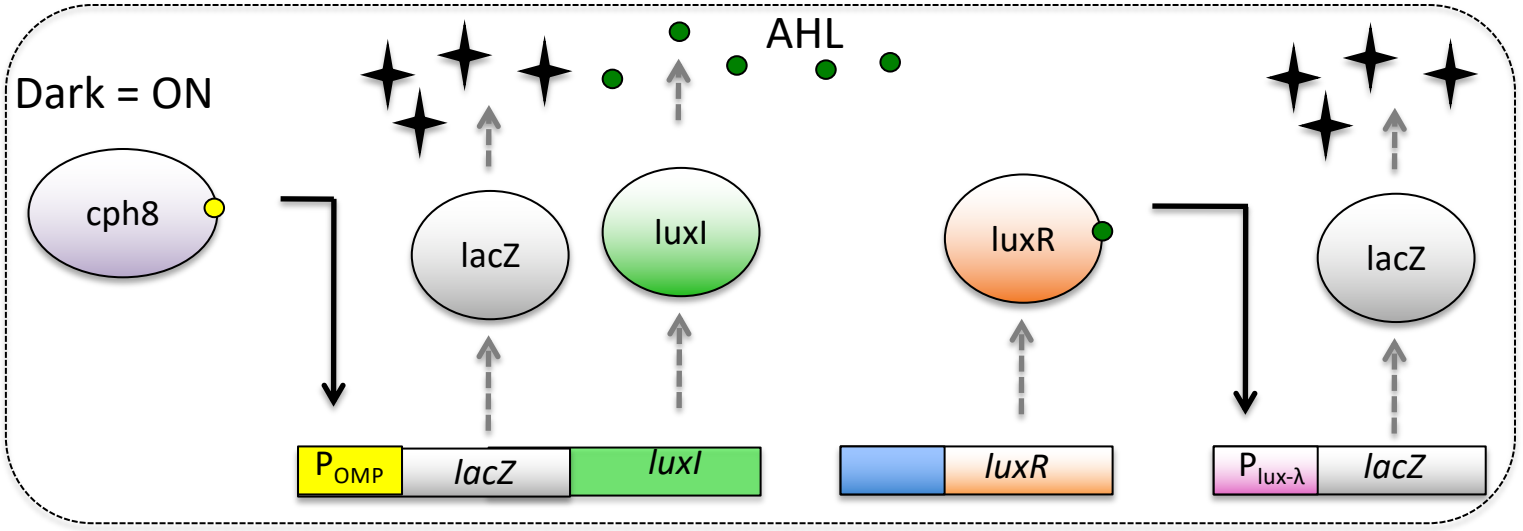


Integrating genetic elements: testing individual modules

Testing communication



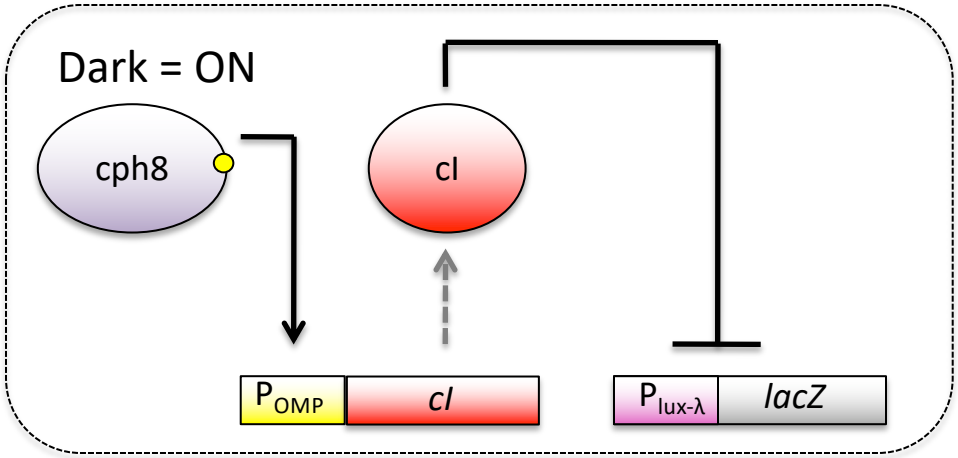
Mask

In vivo

AHL diffuses in
(but not too much)

Integrating genetic elements: testing individual modules

Testing the inverter



Mask

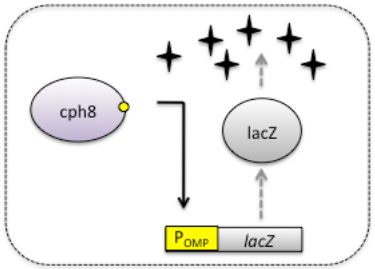
In vivo

Inverting signal = negative image

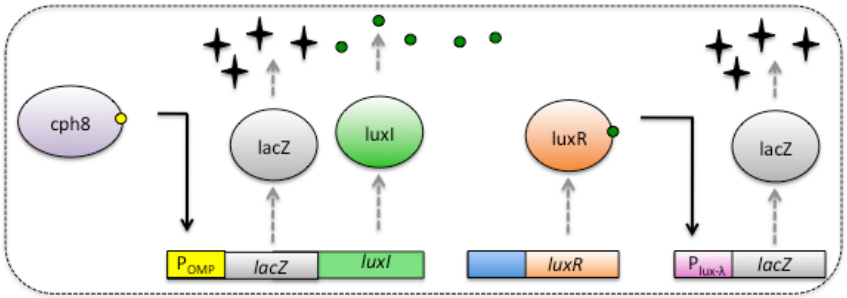
Integrating genetic elements: testing individual modules

Mask

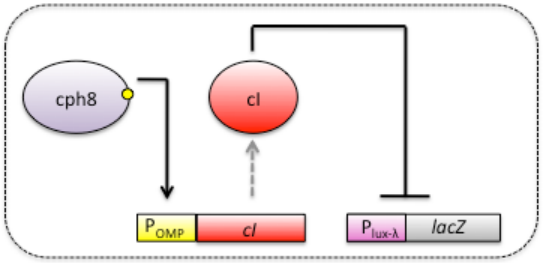
Sensing



Communication

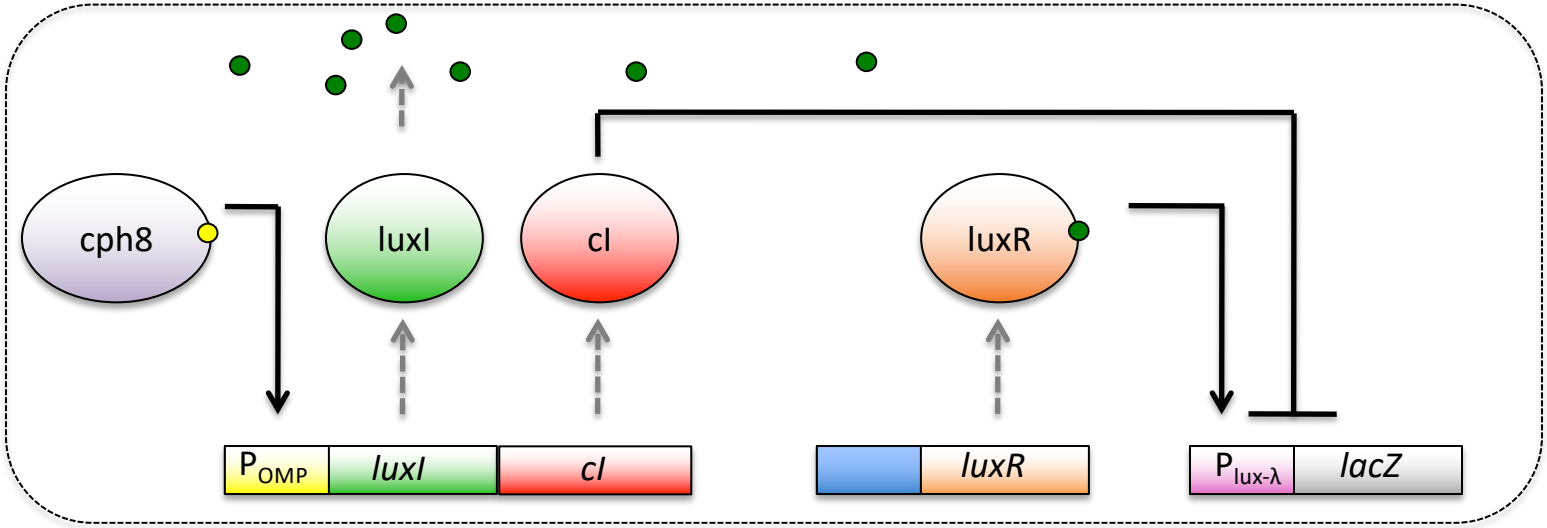


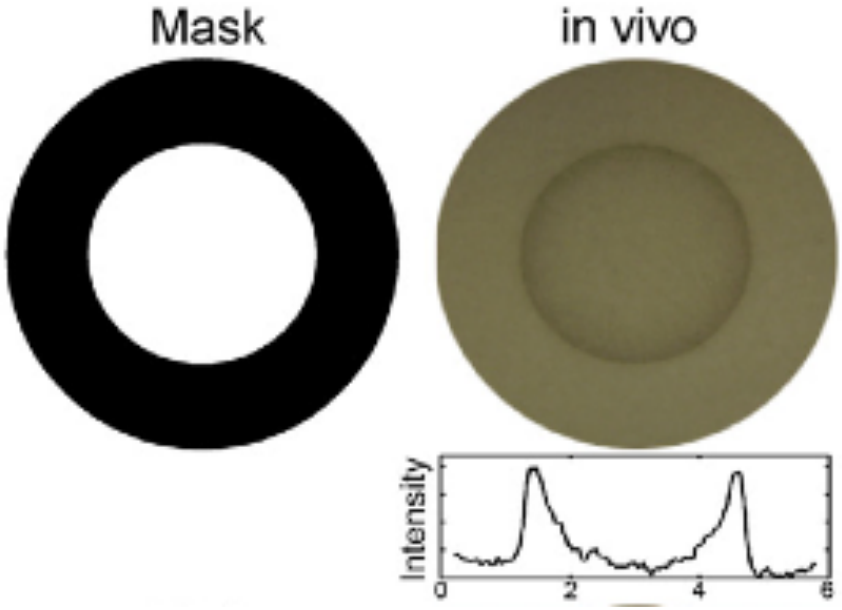
Inverter



Putting it all together....

Bacterial edge detector





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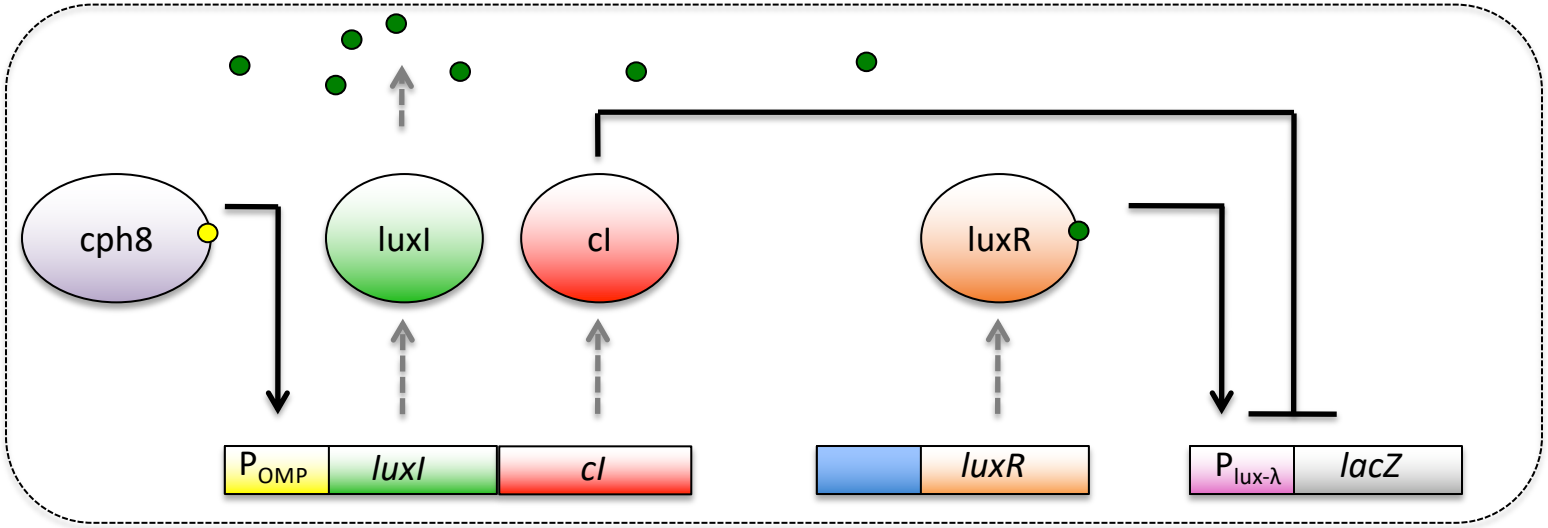
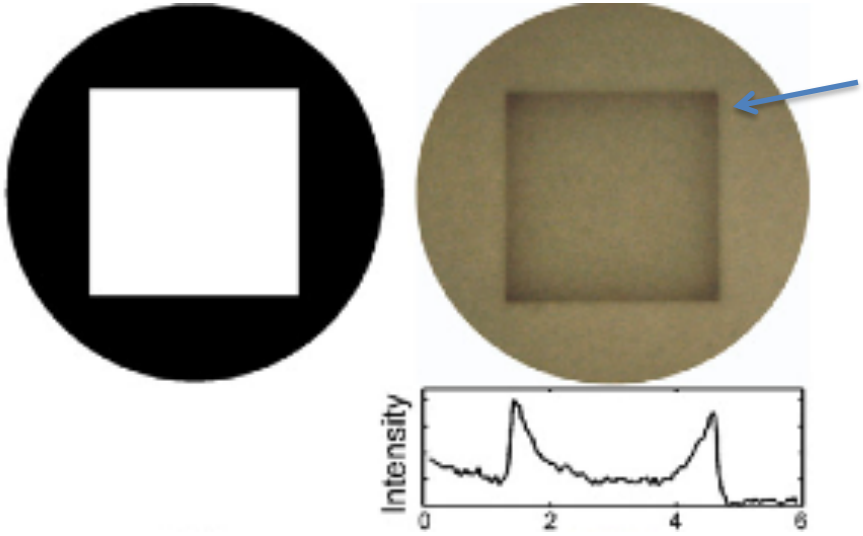
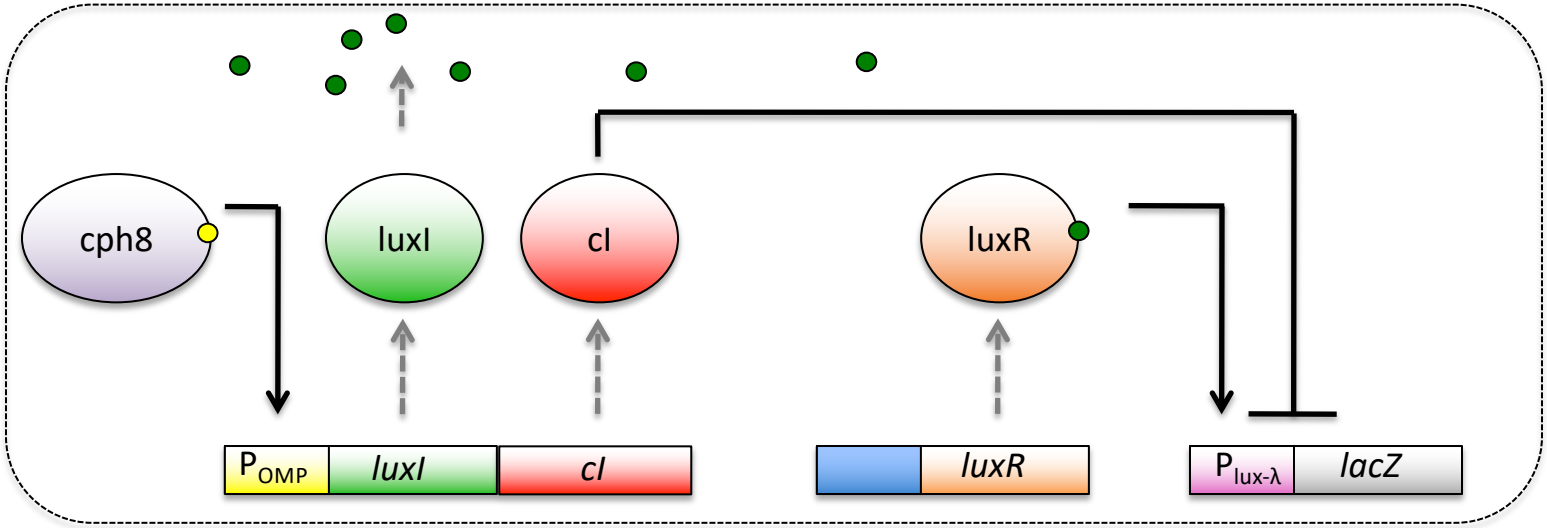
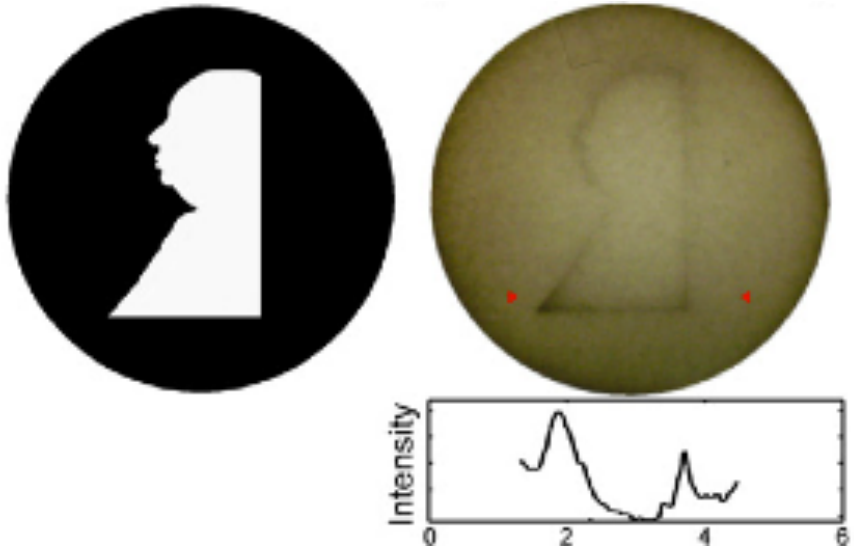




'corner artefact' signals from both edges combine to diffuse further

Bacterial edge detector





Genetic circuit engineering workflow

1. ‘whiteboard’ level design to achieve desired function
2. Design with actual genetics
3. Build (repeat as necessary)
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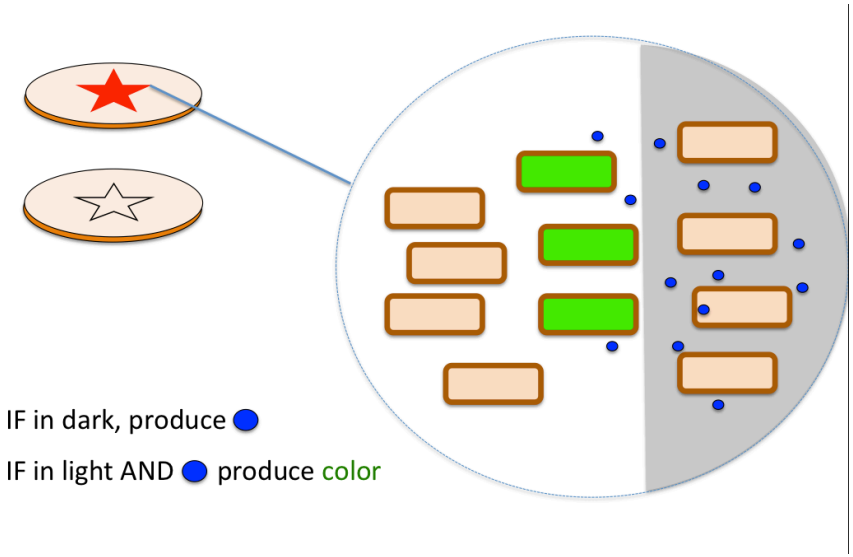

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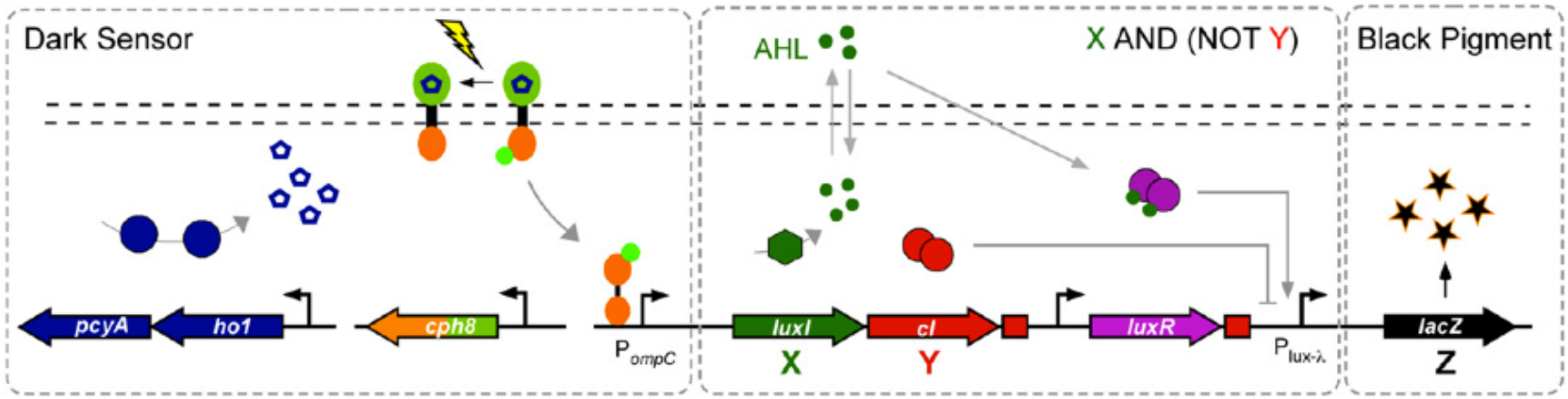

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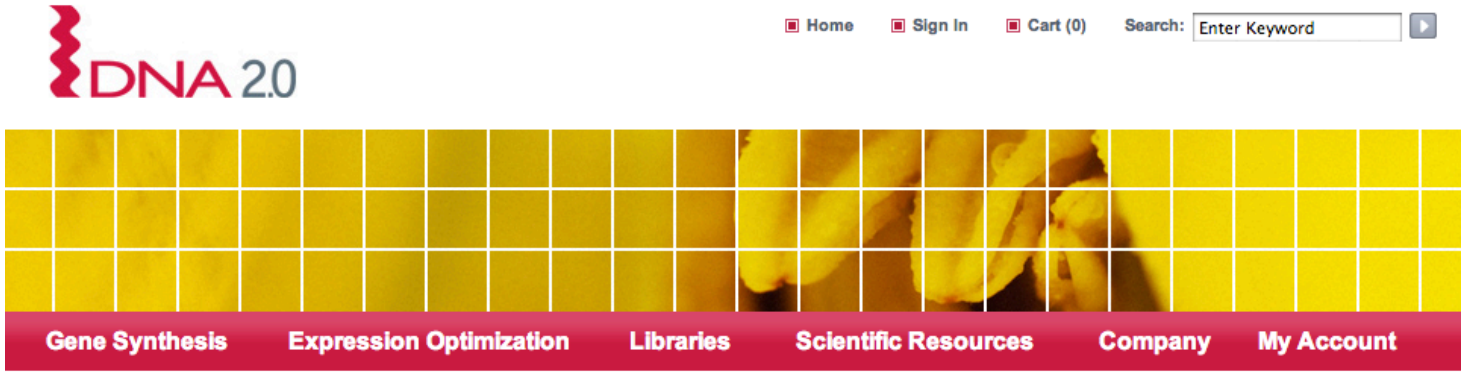

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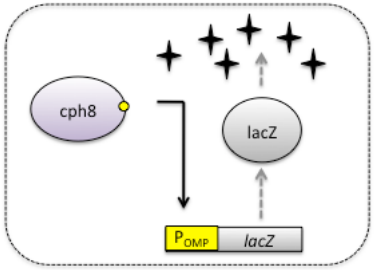

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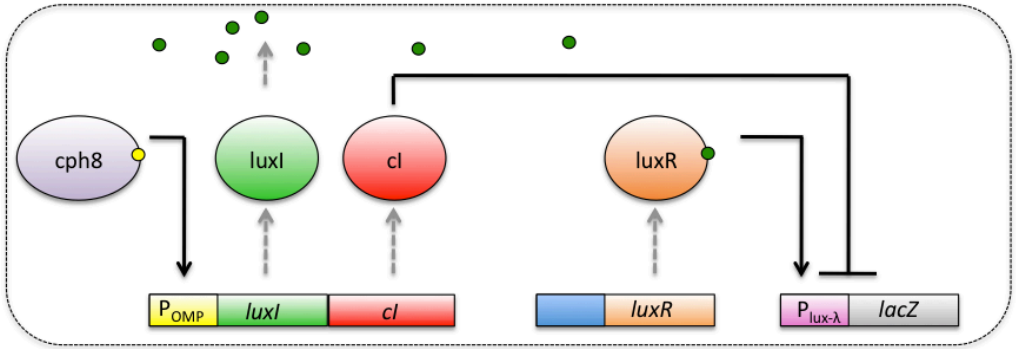
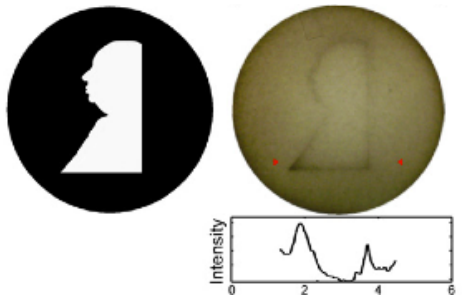
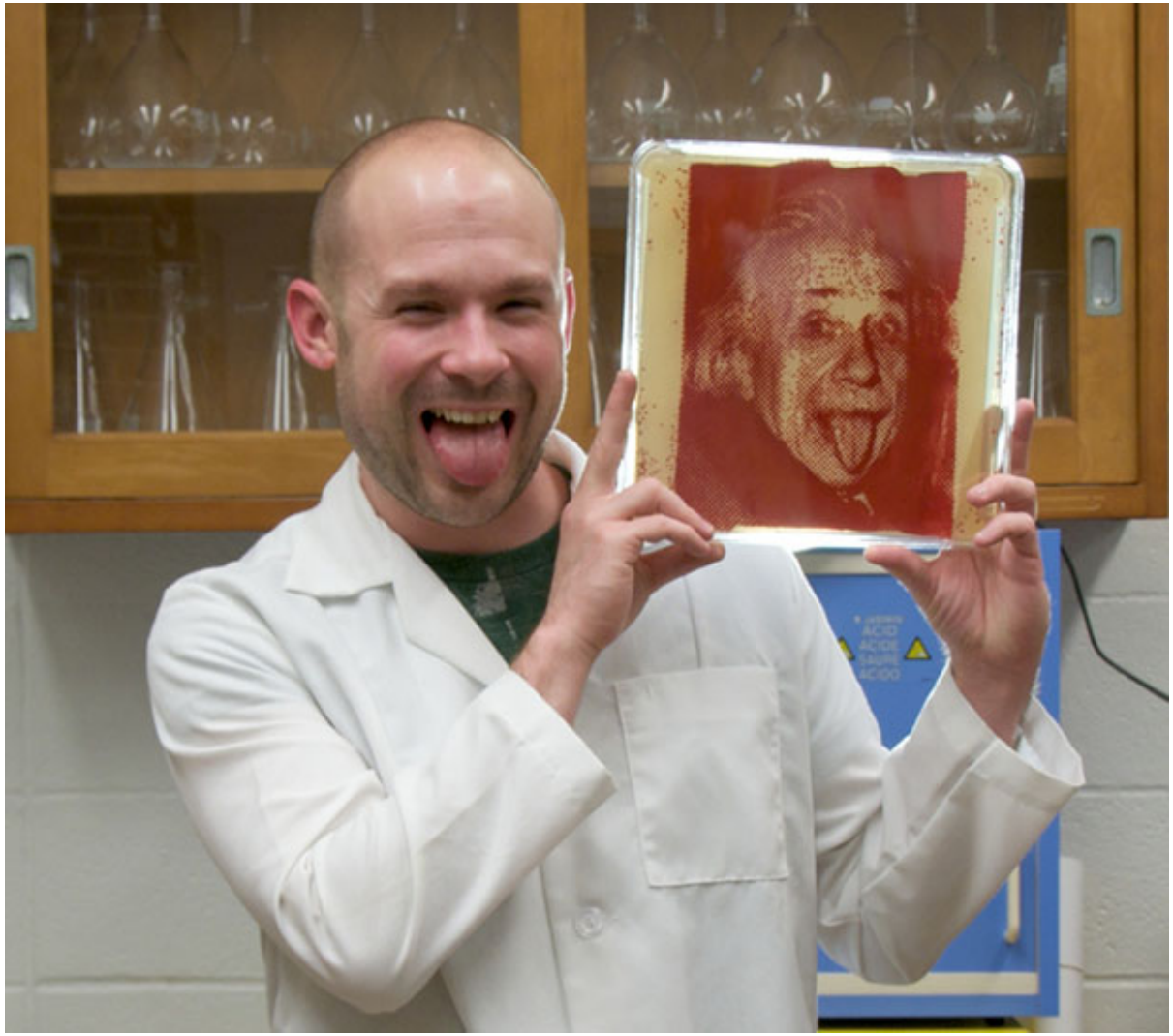




No bacterial selfies – yet.



Optical control: Summary

- **Bacteria (and other organisms) can be engineered to ‘see’ light via expression of receptors**
- **Can integrate light sensing in more complex circuits to program pattern formation**
- **Can interface living and electronic systems for control of gene expression and behavior**

Suggested reading

Primary papers:

Bacterial photography and edge detector

Levskaya et al. Engineering Escherichia coli to see light. *Nature*. 2005 Nov 24;438(7067):441-2.

Tabor et al. A Synthetic Genetic Edge Detection Program. *Cell*. 2009 Jun 26;137(7):1272-81.

Further reading:

Yeast

Milias-Argeitis et al., In silico feedback for in vivo regulation of a gene expression circuit. *Nat Biotechnol.* 2011;29(12):1114-6

Optogenetics: Engineering optical control of biology

- **Aims**

  To present at a circuit level how bacterial photography and edge detection function.

  To introduce brief examples of optogenetics in various organisms.

- **Learning Outcomes**

  To be able to describe bacterial photography and edge detection circuits.