Photoreceptors in synthetic biology

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What?

The photoreceptor

Based on combining a cyanobacterial phytochrome Cph1 with well-studied EnvZ-OmpR system.

For chromphore formation, heme must be converted into phycocyanobilin (PCB). A heme oxygenase (ho1) and a phycocyanobilin:ferredoxin oxidoreductase (PcyA) genes (BBa_I15008 and BBa_I15009) are introduced into the bacterial genome.

With these parts being present, a chimeric Cph1/EnvZ fusion light receptor (BBa_I15010) can be expressed.

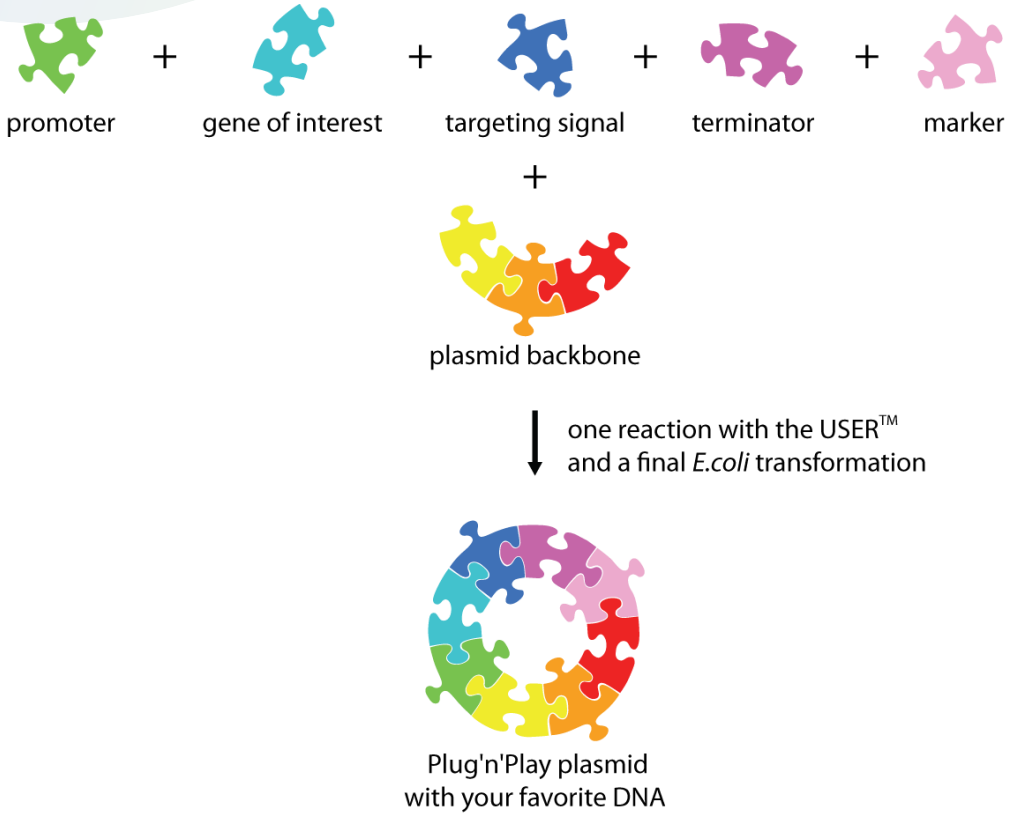
The Cph1 domain responds maximally to wavelengths around 660nm and when active, inhibits the EnvZ-histidine domain.

In the dark EnvZ domain phosphorylates endogenous OmpR which in turn can either activate or suppress transcription depending on promoter type.

The sensor only works properly in a bacteria deficient of natural EnvZ.

How?

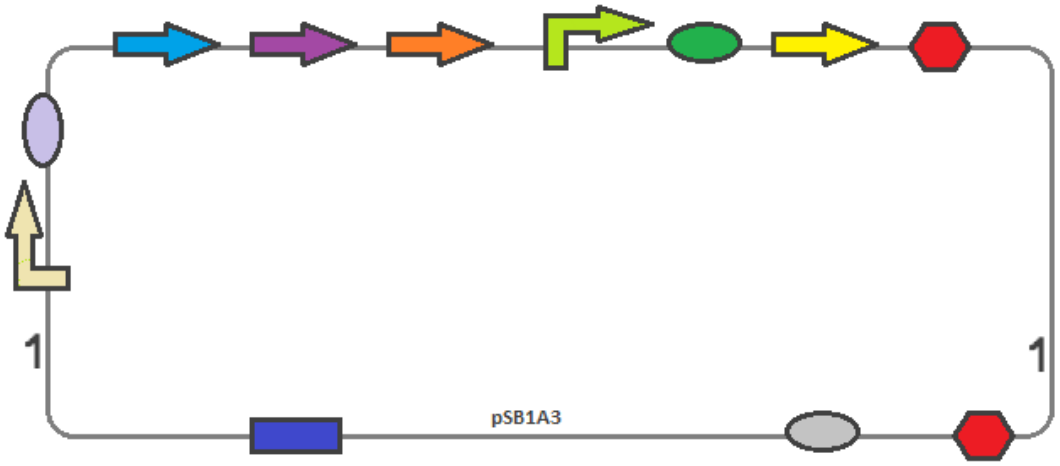
Utilized BioBrick parts



Part number	Description	Notation
BBa_K608003 (BBa_J23104) (BBa_B0032)	Plasmid intermediate consisting of a strong promoter and a ribosomal binding site	strong promoter RBS
BBa_I15008	One of two requisite genes required for the biosynthesis of phycocyanobilin from heme	coding ho1
BBa_I15009	One of two requisite genes required for the biosynthesis of phycocyanobilin from heme	coding PcyA
BBa_I15010	Chimeric Cph1 light receptor/EnvZ protein	coding Cph1/EnvZ fusion
BBa_R0082	Positively regulated promoter, OmpR controlled	regulatory OmpR
BBa_I13504 (BBa_B0034) (BBa_E0040) (BBa_B0015)	Screening plasmid intermediate consisting of a ribosomal binding site, GFP coding gene and a double terminator	RBS coding GFP terminator
pSB1A3 () (BBa_I50020) (BBa_I50020)	High copy assembly plasmid with ampicillin resistance, replication origin and terminators bracketing multiple cloning site	pSB1A3 ampicillin resistance pUC19 replication origin terminator
BBa_G00100	Forward verification primer annealing site (VF2)	VF2
BBa_G00101	Reverse verification primer annealing site (VR)	VR
All parts have assembly compatibility RFC[10].		

Utilized BioBrick parts

- *E. coli* used as the host organism (chassis)
- Selected parts have shown to cause no significant burden to *E. coli* cells
  - Little to no impact on growth



Plasmid assembly with the selected BioBricks.

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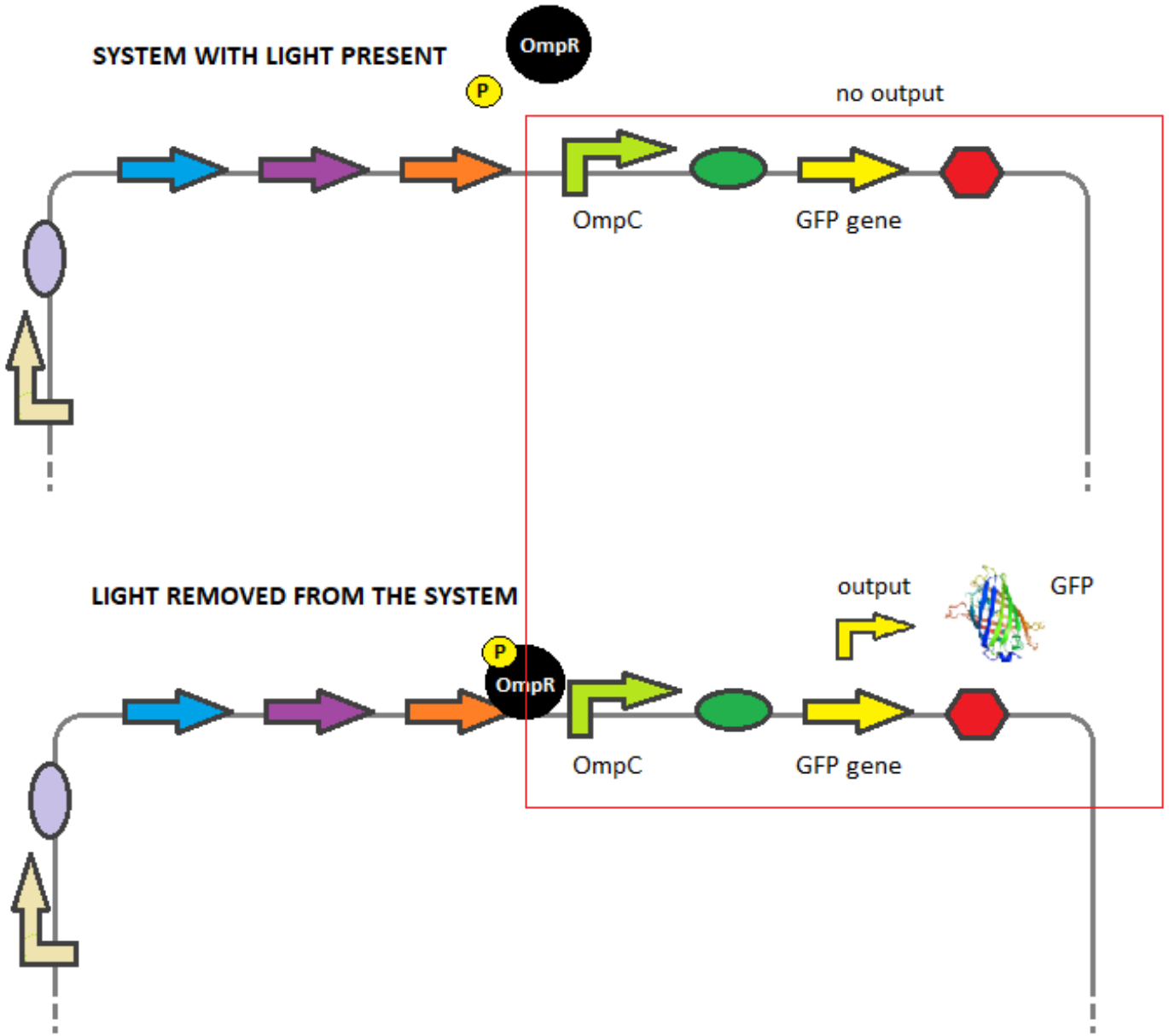
Registry of Standard Biological Parts, The iGEM Parts Registry.

Input/output circuit & truth table



- Transcription activator OmpR is phosphorylated in the absence of light and will bind upstream of OmpC promoter
  - Allows transcription to take place resulting in production of the green fluorescent protein (GFP)
- Activation and repression of the whole system is much more complicated, the presented truth table is based on only the genes in the red box

Light	GFP
1	0
0	1

Truth table for a light regulated system.

Why?

Applications of optogenetically engineered cells in synthetic biology

Optogenetics

- Utilization of light for transferring information and modulating signals in biological systems
- By controlling protein function in target cells with selectable output yield
- Absorption of photons is coupled to a change in functional output

→ Applications in studying neural computation, gene expression and the effects of epigenetic changes

Building logic gates and complex layered circuits (Liu et al., 2018)

- Light-controlled activation and deactivation of CRISPR
  - Enables light control over genes on the chromosome
  - gRNAs are transcribed in response to light and then combined with dcas9 for redirection of metabolic flux
- Bacterial 3D printer
  - Immobilizing bacteria in gels and using intersection of laser beams to trigger gene expression (Paris-Bettencourt 2017 iGEM team)

Therapeutic applications of light-controlled mammalian cells (Mansouri et al., 2019)

- Blood glucose homeostasis in diabetic mice can be monitored and controlled
  - Light-dependent circuit for insulin secretion in mouse pancreatic β-cells by functionally expressing ChR2 in β-cells
  - Blood glycose levels were successfully reduced via blue light-controlled insulin expression
  - Can be controlled though a Bluetooth-compatible glucometer
- Delivery of optogenetic channels to retinal cells that suffer from retinitis pigmentosa (an inherited retinal degeneration disorder) in order to re-sensitize cells to light
  - A native signaling pathway within retinal cells is activated by the engineered photoreceptor
  - Successfully restores vision in blind mice

References

- Registry of Standard Biological Parts, The iGEM Parts Registry. http://parts.igem.org/ [accessed 24/03/2023]
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Thank you!