THE CALCIUM KIT

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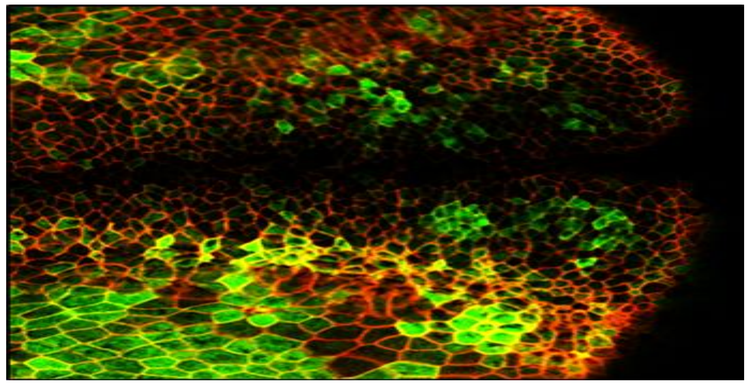
Introduction

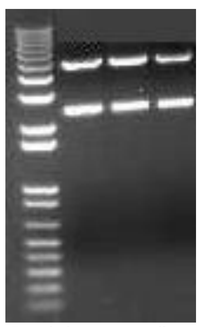
Calcium is a ubiquitous eukaryotic signaling molecule that regulates a wide array of cellular processes including fate determination, excitability, motility and adhesion, and regeneration. While there are numerous methods for monitoring calcium activity through both chemical and genetically encoded indicators, there is currently a lack of tools for higher-level measurement such as *in vivo* calcium spike counting. Because developing advanced circuits is very challenging in eukaryotic systems, the William & Mary iGEM team aimed to create a toolkit for inducing, monitoring, and measuring calcium activity in bacteria. This set of foundational tools will allow future synthetic biologists to develop more sophisticated genetic systems for studying calcium.



Visualization of transient calcium activity in a developing *Xenopus laevis* embryo. Cells are outlined with memRFP (red) and calcium activity is indicated by GCaMP (green). This is an example of current calcium visualization techniques, but is not capable of recording calcium history.
Photo courtesy of Wendy Herbst.

Methodology

We obtained ChR1 from Addgene (pcDNA3.1/VChR1-EYFP), and CatCh (ChR2-L132C-T159C-mcherry) from the lab of Dr. Peter Hegemann. Both ChR1 and CatCh had internal PstI cut sites, which we removed through site-directed mutageneses (SDM): G2044T in ChR1; G843T and G1968T in CatCh. The SDM protocol entails PCR amplification of the coding region using primers containing the designed mutation, followed by a Kinase-Ligase-DPN1 (KLD) reaction to circularize the PCR product, and subsequent digestion with EcoRI and PstI for insertion into a BioBrick backbone.



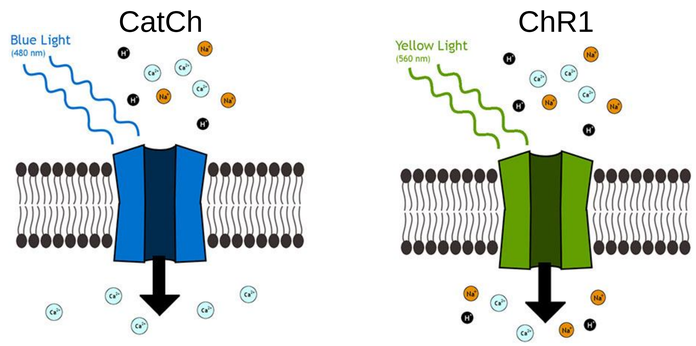
Restriction digest confirmation of ChR1 BioBrick clones!

Objectives

We planned to create three different classes of parts that: induce calcium influx in bacteria; indicate the presence of calcium; and record the number of calcium spikes.

1. Induce calcium influx in bacteria:

Channelrhodopsins are light-activated cation channels that, when excited, allow positively charged ions to flow from the extracellular space into the cell. There are two naturally existing channelrhodopsins: channelrhodopsin-1 (ChR1) and channelrhodopsin-2 (ChR2). Additionally, there exist versions, including CatCh, that have been optimized for specific situations.



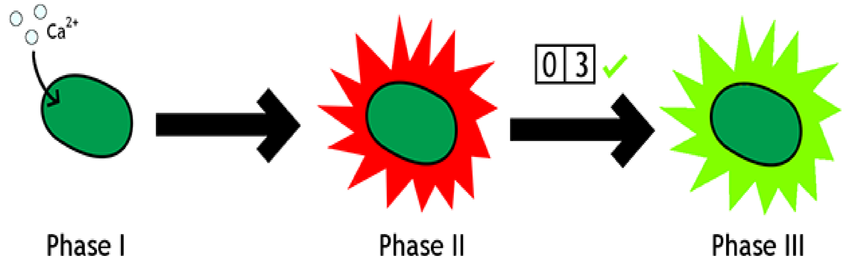
A choice in channelrhodopsins. Calcium Relocating Channelrhodopsin (CatCh) is activated by blue light (480nm) and is calcium-specific. ChR1 is activated by yellow light (560nm) and is cation-specific.

2. Indicate the presence of calcium:

Genetically Encoded Calcium Indicators (GECIs) are fusions of a fluorescent protein and calmodulin. The fluorescent protein is inactive until calmodulin binds to calcium. This induces a conformational change that activates the fluorescent molecule, allowing for the monitoring of changes in calcium levels.

3. Record the number of calcium spikes:

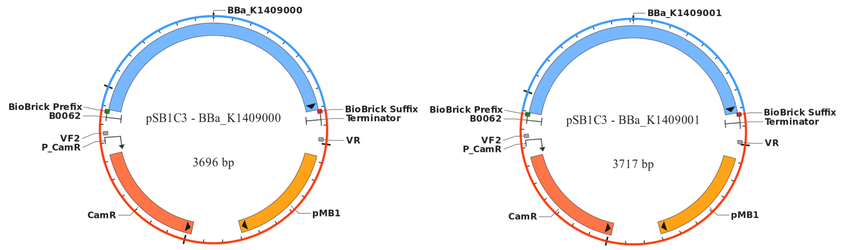
A calcium-dependent promoter would activate a counting circuit that, after a specified number of spikes, produces a reporter molecule.



The three phases of calcium manipulation in bacteria. **Phase I:** Ca^{2+} concentration in the cell is increased through activation of a calcium-specific ion channel. **Phase II:** Increase in calcium concentration activates a genetically encoded calcium indicator (GECI). **Phase III:** After a user-defined number of spikes in calcium concentration, transcription of a different fluorescent indicator is activated.

Parts Submitted

ChR1 (BBa_K1409000)
Channelrhodopsin-1 is a light-gated ion channel that is activated by light of approximately 560nm (yellow light). ChR1 is a non-specific cation channel, allowing the influx of ions such as calcium into cells.
CatCh (BBa_K1409001)
Calcium Relocating Channelrhodopsin (CatCh), is a ChR2 variant with increased permeability to Ca^{2+}.



Plasmid maps of submitted parts. Parts BBa_K1409000 and BBa_K1409001 are ChR1 and CatCh, respectively.

Conclusions

We successfully created and submitted two new parts: ChR1 (BBa_K1409000) and CatCh (BBa_K1409001). These two channelrhodopsins have been designed to meet the BioBrick RFC10 standard, ensuring that these parts can easily be used by future teams.

Future directions

The next step towards completing a set of tools to enable the study of calcium dynamics in bacteria is to build BioBricks for reporting and measuring calcium activity. We are confident that the introduction of these essential parts will enable future iGEM teams to create genetic circuits for monitoring calcium in bacteria, and eventually eukaryotes.

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