## Background

Oysters are an integral piece of the Chesapeake Bay’s ecosystem. They are responsible for filtering contaminants, such as heavy metals, toxins, and pollutants, out of the water and serve as a food source for much of the eastern seaboard. However, due to various issues, the oyster population has declined sharply from previous decades. One of the reasons for this decline is the pathogen Perkinsus marinus, which causes Dermo disease.

Perkinsus marinus is a unicellular protist that invades oyster hemocytes by inducing phagocytosis via CvGa1, a galectin secreted by the hemocyte that normally serves to signal the presence of food and/or pathogens. The dual binding of CvGa1 to the pathogen and hemocyte triggers phagocytosis. Once inside the cell, Dermo resists the reactive oxygen species (ROS) system that would normally quell infections. P. marinus then proliferates, lysing the hemocyte and infecting the host oyster.

Currently, no real-time detection methods for Dermo exist. We thus decided to create a biosensor capable of doing so. Due to complications with producing CvGa1 capable of being expressed by E. coli, our group used a model galectin known as Bovine Galactin 1 (BtGal1). Our three step process is illustrated on the left. We cloned a bacterial construct which would display BtGal1 outside of the cell in order to bind to the pathogen. A subsequent signaling cascade leading to fluorescence would be easily identifiable using convention microscopy methods.

Steps for preliminary proposed detection method:
1. Arabinose induces OmpA-BtGal1 protein which integrates into the cellular membrane (TOP)
2. These cells are stained via nuclear stain for easy detection (MIDDLE)
3. Cells are placed in an environment with the pathogen (MIDDLE)
4. Fluorescently labeled bacteria bind to Dermo in high quantities and are easily identifiable using fluorescence microscopy (BOTTOM)

## Methods

OmpA (BBa_K103006) was excised from pSB1A2 via EcoRI and PstI and ligated into pSB1C3 to make BBa_K1489002. Site-directed mutagenesis was used to convert SacI site on BBa_K1489002 into Kasi site, generating BBa_K1489003. BBa_K1489000, BBa_K1489004 were cloned via Gibson Assembly using BioBricks and gblocks (IDT). Site-directed mutagenesis was used to remove an undesired EcoRII site present in BtGal1.

BtGal1 and OmpA-BtGal1 were initially expressed under Arabinose induction (0.2%, 22 hours). Western blots using anti-His were conducted in order to confirm the presence of his-tagged proteins of the correct molecular weight.

Next, BtGal1 was induced at high quantities for binding assays. E. coli were grown to proper O.D. then induced with 0.1% arabinose for 6 hours. Cell pellet was lysed using French press and purified on a Cobalt affinity column. Fluorescence binding assay test was subsequently performed on purified protein in order to confirm the function of BtGal1 and characterize binding affinity.

## Characteristics

### BioBrick Characterization

**Western blot (anti-His)**

**Fluorescence assay of BtGal1**

**Fluorescence binding assay for live bacterial detection**

### Human Practices

**Chesapeake Bay**

- **Economics**
- **Policy**
- **Education**

**Dangerous forces in the Chesapeake Bay:**

- **Fish farming**
- **Land use and development**
- **Transportation**

**Chesapeake Bay Foundation**

- **Department of Natural Resources**
- **Oyster Farm**

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**References:**

3. Källberg L, Deng CX, Nieminen T, et al. The BtGal1 and BtGal1 constructs were successfully expressed.
4. We found that BtGal1 (BBa_K1489000) is functional via binding assay with lactose.