E. coli

**Red Light-regulated system**
For cph1 to sense light in E. coli, it requires phycocyanobilin (PCB). We therefore introduced two PCB-biosynthesis genes, **ho1** and **pcyA** from *Synechocystis*, converting heme into PCB. With PCB, cph1 serves as a red-light absorbing chromophore that is inactivated under red light and activated without red light.

**Temperature-regulated system**
The secondary structure of RNA thermometer masks the SD sequence and prevents ribosome binding, therefore initiation of translation is forestalled. When the temperature reaches 37°C, the base-pairing nucleotides lose their binding forces and exposes the SD sequence to ribosome. In this way, gene expression can be regulated on the RNA level by temperature.

**sRNA-regulated system**
A non-coding sRNA would recognize a special sequence called the sRNA-binding site. Once the sRNA binds to the sRNA-binding site, it would hinder ribosomes from binding and initiating translation. Here, we designed two artificial sRNAs, each modified from different paper. We also designed a RBS specifically targeted by our sRNA. By adding this RBS to the upstream of any desired gene, the gene can be regulated by our sRNA. Unlike the regulated-systems popularly used in iGEM projects, sRNA-regulated system seems more efficient.

**Abstract**
The majority of the previous iGEM projects focused on expressing certain genes to achieve a specific task such as sensing a certain phenomenon. This year, we took a step forward in building a system that is capable of regulating multiple genes in *E. coli*. The system, so-called E.colightuner, is built by three regulated-systems which used noninvasive factors such as red light and temperature to create different conditions under which *E. coli* can express different genes. We also introduced a new sRNA-regulated system that hasn’t been widely applied in iGEM before.

**Human Practice**
We hosted a four-day iGEM conference which we invited NVMU, NTU, SITU, ZIU, and HKU to participate and we also held Bio-camp for high school students, extending synthetic biology. We’re eager to share our ideas to other brilliant teams, and we learned from them as well. Besides, we also introduce our project to the public and make tissue culture as souvenirs for the listeners.

**Contributions**
- **New BioBrick**: rRBS: sRNA target RBS -K1017202
- **sRNA**: a functional sequence inhibit promoter -K1017404
- **Improvement**: Correct cph8 registry part -K1017301
- **Increase the convenience of red light sensing system**

**Mechanism of E.colightuner**

**Red Light Induced Circuit**
First, we did an experiment that tested the fluorescence at different temperature and different time. Then we input these sets of data to ANFIS system to model the graph like Figure 15.

**Application**
By replacing the fluorescent proteins with plant hormones, we can offer multiple hormones secreted by *E.coli* in different growing stages to help the plants grow.

**Modeling**

**Figure 15**
- Input1: time (hr), Input2: Temperature (°C), Output: Normalized expression (AU)
- The modeling of the strength of P_rbs

We simulated the P_red expression versus time using the data of P_rbs and P_lac. After getting the experimental data, we can fit in this model and modify it for predict the result better. (see fig 16.)

**Figure 16**
- Model curve

**Figure 12**
- Different gene expressions of E.coli.

**Figure 13**
- The above is our device to culture plants.

**Figure 14**
- Add different frequencies of light or use different intensity of red light and temperature to regulate more genes.