A Dual-Input Reporter System in *E. coli*

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**Abstract**

We constructed a novel molecular AND gate in *E. coli*. An AND gate requires two positive inputs to generate a single output. Either positive input without the other does not generate an output. Our AND gate expresses a reporter in the presence of both ROS and high temperature. We selected an OxyR-responsive promoter (HemH) and a thermo-sensitive riboswitch (derived from Listeria) as detectors for ROS and temperature, respectively. The OxyR-responsive promoter is used to drive transcription of the Listera thermo-sensitive riboswitch coupled to a Cre/Lox system which, when activated, removes a double-terminator sequence and allows constitutive transcription of the reporter molecule. This system may be further modified and adapted to various applications, including early detection of colon cancer, as cancerous cells produce excess ROS and heat.

**AND gate Mechanism**

Our full AND gate contains one extra function more than the simple model described in figure A below. We use a Cre/Lox system in order to achieve a sustained (constitutive) level of output “Y” after possibly transitory levels of inputs “A” and “B” — see figure B below. To accomplish constitutive reporter expression, we put our reporter under the control of a constitutive synthetic promoter. As can be seen in the above figure, inputs “A” and “B” allow transcription and translation of the Cre protein. Cre is a site-specific recombinase that loops out any DNA between two identical 34 base sites called Lox sites. In our final construct, we cloned two Lox sites that sandwiched two transcriptional terminators in between the constitutive promoter and the reporter. While the Lox sites containing the double-terminators are present, the reporter is silent. When the AND gate allows transcription of Cre, Cre removes the Lox sites and the double-terminator, allowing the reporter to be constitutively expressed from that point on.

**Thermosensor Results**

Using the wild-type thermo-sensitive riboswitch isolated from Listeria as our template, we then found, through mutagenic PCR, two thermosensors with narrower temperature ranges, and four thermosensors with increased or decreased expression after melting. We are especially pleased with the narrowed temperature ranges of BBa_K619890 and BBa_K619891, which demonstrate the most narrow temperature sensitivity of any known riboswitches from our literature research.

**Methods Used in Thermosensor Tests**

Creation of our wild-type thermosensor involved annealing of two oligos. We optimized the SD sequence for that of *E. coli*. After cloning this sequence into *E. coli* and verified that it was working, we used this construct for our template to perform mutagenic PCR for additional thermosensors. Mutagenic PCR involved an unstable taq-polymerase and varied ratios of purines and pyrimidines, this resulted thermosensors with optimized temperature ranges.

**Screen**

We screened through thousands of colonies to select for possible optimized thermosensors. We grew colonies at 30°C and 37°C, then transferred plates to 37°C range. Thus far, we have been unable to find other examples of thermosensors that loops out any DNA between two identical 34 base sites called Lox sites. In our final construct, we cloned two Lox sites that sandwiched two transcriptional terminators in between the constitutive promoter and the reporter. While the Lox sites containing the double-terminators are present, the reporter is silent. When the AND gate allows transcription of Cre, Cre removes the Lox sites and the double-terminator, allowing the reporter to be constitutively expressed from that point on.

**Pre-screen**

Plate left: Plate containing colonies with mutagenic PCR created thermosensor mutants. Plate was grown at 37°C, note variation in LacZ expression.

**Verification**

We verified our original screen by streaking specific hour shifts against our verified wild thermosensor results. See photos from this screen below:

**AND Gate Proof of Concept**

We grew colonies containing our AND gate. Only when plated in the presence of both arabinose and heat was LacZ expressed.

When plated in conditions lacking either of the two inputs, there was no detectable LacZ activity.

**Table:**

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<tr>
<th>Predicted Hairpin Structure</th>
<th>Temperature Range and Part Number</th>
<th>Colonies at 30°C</th>
<th>Colonies at 37°C</th>
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<th>Predicted Hairpin Structure</th>
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**References**