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

**Abstract**
An AND gate is a logic gate that requires two simultaneous positive inputs to generate a single output. Our AND gate expresses a reporter in the presence of both reactive oxygen species (ROS) and high temperature. We selected a SoxR-responsive promoter (pSoxS) and engineered a thermo-sensitive hairpin loop (derived from *Listeria*) as detectors for ROS and temperature, respectively. This system may be further modified and adapted to various applications, including early detection of colon cancer.

**AND Gate Mechanism**
Our AND gate is a molecular implementation of the simple model in figure A below. In the presence of ROS, the SoxR protein (naturally present in *E. coli*) is activated by redox cycling agents and binds pSoxS, activating transcription. The resulting mRNA folds into a hairpin loop which buries the Shine-Delgarno RBS and the ATG start codon, preventing translation. Above a temperature threshold, the hairpin loop unfolds and allows translation of the reporter gene. Our reporter is LacZ, which turns blue in the presence of X-gal. Only in the presence of both inputs (ROS and heat) will the reporter be expressed.

**Thermosensor Screen**
We randomly mutagenized a wild-type thermosensor isolated from *Listeria monocytogenes*. We screened the products for thermosensors with a narrower on/off temperature range than the wild-type. We found four with greater contrast at the wild-type’s natural range of 30–37°C and two with a much narrower temperature range of 35–37°C. We are especially pleased with the narrowed 2-degree ranges, which are more specific than any thermosensor we have found in the literature.

**Thermosensor Data**

<table>
<thead>
<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>
</tr>
</thead>
<tbody>
<tr>
<td>30-37°C</td>
<td>BBA_K619889</td>
<td></td>
<td></td>
</tr>
<tr>
<td>30-37°C</td>
<td>BBA_K619893</td>
<td></td>
<td></td>
</tr>
<tr>
<td>30-37°C</td>
<td>BBA_K619894</td>
<td></td>
<td></td>
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</tbody>
</table>

**AND Gate Data**
To demonstrate proof of concept, we included the arabinose-inducible promoter pBAD as one of the two inputs. The top right plate is blue because both inputs were present.

**Results**
We were very pleased with the variety of thermosensors we produced, especially those that changed within the 35–37°C range. Thus far, we have been unable to find other examples of thermosensors in the literature with such a narrow and physiologically relevant temperature range.

The AND gate proof of concept was successful. Not only did we produce two functioning logic gates, but we also demonstrated the ability to interchange promoters, which offers flexibility to bioengineers in designing unique AND gate-based biosensors.

**pSoxS Data**
We used a part characterized by team UNICAMP-EMSE Brazil this year to detect ROS. Our *E. coli* did not express excess SoxR protein, and the system still responded well to Paraquat. The graph at right displays the fluorescent response.

**References**