***Supplementary Material***

1. **List of Supplementary Data**

Table S1: iGEM Registry IDs for sequences and constructs used in this study

Figure S1: Induction over time of receivers with sender supernatant

Figure S2: Sender-Receiver induction map based on results from assays with cell-free, HSL-enriched liquid media

Figure S3: Sender-Receiver induction map based on results from assays on solid agar

1. **Supplementary Tables**

**Supplementary Table S1.** iGEM Registry IDs for sequences and constructs used in this study. “Antiquity” indicates that no specific team is known to have contributed the DNA sequence to the Registry. Entries can be accessed at <http://parts.igem.org/>. Normalized (GFP/OD600) GFP signal data from 96-well plate time course experiments with synthetic HSLs and HSL-enriched Sender media are available for each Receiver in Microsoft Excel format. To access the data, please visit <http://parts.igem.org/>, search for the iGEM Registry part number (e.g. BBa\_K2357000), navigate to “experience”, and find the Excel file under “Applications of…” (a) BBa\_K2357021 is a derivative of BBa\_F2620. An ORF encoding GFP-mut3b (BBa\_E0040) was cloned downstream of BBa\_F2620.

|  |  |  |
| --- | --- | --- |
| **Part name** | **iGEM Registry part number** | **Contributing iGEM Team** |
| LasR Receiver | BBa\_K2357000 | iGEM17\_Arizona \_State |
| TraR Receiver | BBa\_K2357028 | iGEM17\_Arizona \_State |
| AubR Receiver | BBa\_K2357014 | iGEM17\_Arizona \_State |
| BjaR Receiver | BBa\_K2357007 | iGEM17\_Arizona \_State |
| LuxR Receiver(a) | BBa\_K2357021 | iGEM17\_Arizona \_State |
| RpaR Receiver | BBa\_K2357001 | iGEM17\_Arizona \_State |
| RhlR Receiver | BBa\_K2357002 | iGEM17\_Arizona \_State |
| RpaI  | BBa\_K1421006 | iGEM14\_CAU\_China |
| BraI  | BBa\_K2033004 | iGEM16\_Arizona\_State |
| RhlI  | BBa\_C0170 | Antiquity |
| BjaI  | BBa\_K2033002 | iGEM16\_Arizona\_State |
| EsaI  | BBa\_K1670004 | iGEM15\_Manchester-Graz |
| LuxI  | BBa\_C0161 | Antiquity |
| SinI  | BBa\_K2033008 | iGEM16\_Arizona\_State  |
| AubI  | BBa\_K2033000 | iGEM16\_Arizona\_State |
| LasI  | BBa\_C0078 | Antiquity |
| CerI  | BBa\_K2033006 | iGEM16\_Arizona\_State |

1. **Supplementary Figures**



**Supplementary Figure S1**. Induction over time of receivers with sender supernatant. Average GFP/OD600 over time. Receivers cells were diluted to OD600 = 0.4 in fresh LB and inoculated with filtered, HSL-enriched supernatant (HSL-s). Percent HSL is calculated based on the fraction of HSL-enriched supernatant in the total volume of treated Receiver culture (see Figure 4). Cells were incubated at 37℃ with constant orbital rotation with GFP (excitation 485, emission 515). GFP and OD600 were measured every 10 minutes over 8 hours. Error bars represent standard deviation of three wells.



**Figure S2**. Sender-Receiver induction map based on results from assays with cell-free, HSL-enriched liquid media. Cells are represented as nodes (ovals) linked by edges that are formatted to represent the sensitivity of each Receiver (green) to the HSL-enriched supernatant produced by each Sender (pink). High, medium, and low sensitivity represent inductions where 10%, 25%, or 50% HSL-enriched medium significantly increased GFP signal from a Receiver compared to mock-enriched medium (*p* < 0.05, Student’s one-tailed t-test). Sets 1 and 2 (boxes) represent Sender/Receiver pairs that are expected to operate without crosstalk. Set 2 is predicted to exhibit no crosstalk at low HSL concentrations (i.e. 10% HSL-enriched medium).



**Figure S3**. Sender-Receiver induction map based on results from assays on solid agar. As in Figure S2, Sender (pink) and Receiver (green) cells are represented as nodes (ovals) linked by edges that are formatted to represent the strength of induction. Here, induction strength is represented by relative induction distance across a lawn of Receiver cells, which was determined as described in Figure 5. Strong, medium, and low induction strengths, are classified by distance thresholds shown in the legend (bottom left). Shaded boxes indicate orthogonal sets that are also predicted by induction with HSL-enriched media in liquid cultures (sets 1 and 2 from Figure S2).