Cell-free and *in vivo* characterization of Lux, Las, and Rpa quorum activation systems in *E. coli*

Supporting Information

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Materials and methods
TX-TL:
TX-TL was prepared using BL-21 Rosetta2 cells (Novagen) as previously described. All TX-TL reactions were conducted at 5uL final volume with a maximum of 25% final volume consisting of DNA. TX-TL extract was 33% final concentration, TX-TL buffer 42% final concentration, and DNA 25%. Any extra volume was filled with Nuclease-Free Water (Ambion). All liquid transfers for TX-TL preparation were performed by an acoustic liquid handling robot, the Echo 525 (LabCyte). After liquid transfer was complete plates were spun at 2000g for 2 minutes to spread the TX-TL across the well. Plates were then sealed with a polylofein seal (Thermo Fisher Scientific) to prevent evaporation. Plates were read in a BioTek Synergy H1M incubated at 29°C with excitation wavelength of 485nm and emission wavelength of 515nm for deGFP. All plasmids used for TX-TL were purified using NucleoBond Xtra Midiprep kit (Macherey-Nagel). AHLs (Sigma Aldrich) were suspended in DMSO and were added such that the final concentration of DMSO in each reaction was fixed at 1%. All TX-TL experiments had negative controls for both auto fluorescence of the TX-TL (wells containing only extract, buffer, and water, with no DNA or AHL added), and for background or “leak” of the DNA (TX-TL containing extract, buffer, water, DNA, but no AHL). For all experiments shown we subtracted the auto fluorescence of TX-TL from the time traces and displayed the “leak” of the DNA clearly in Figure 1a (0nM AHL condition). All TX-TL experiments were conducted at a final concentration of 1% DMSO, the agent used to solubilize the AHL, to control for any confounding variable introduced by the DMSO itself. All TX-TL negative controls were run in quadruplicate for each experiment.

In vivo:
JM109 Mix & Go cells (Zymo Research) were co-transformed with a plasmid constitutively expressing the transcription factor on p15a origin containing chloramphenicol resistance and a plasmid with the AHL promoter regulating GFP expression on a ColE1 origin containing kanamycin resistance. Three colonies from each plate were inoculated into LB containing chloramphenicol (34ug/mL) and kanamycin (50ug/mL) overnight and grown until saturation. Cultures were then diluted 1:100 fold into M9CA (TekNova) containing chloramphenicol (34ug/mL) and kanamycin (50ug/mL) and grown until mid-log phase (0.3-0.6 OD600). Cultures were then diluted into M9Ca containing chloramphenicol (34ug/mL) and kanamycin (50ug/mL) and 1uM of the appropriate AHL, or 0.1% DMSO as a negative control. 150uL of culture was grown in 96-well flat-bottom round-well plates (PerkinElmer). Cells were grown in a BioTek Synergy H1m with constant linear shaking and 37°C incubation. An excitation wavelength of 485nm and emission wavelength of 515nm for deGFP was used. 600nM absorbance was used for calculating cell density. As with the TX-TL experiments, all in vivo experiments also were conducted at the same DMSO concentration to avoid a confounding variable. Background values were calculated for each of the nine strains by running DMSO only (no AHL) conditions. These background values were then subtracted from each trace. All in vivo negative controls were run in triplicate for each experiment.

Data Analysis:
Data analysis was performed using custom Python scripts. All raw data and code used in this manuscript is available at github.com/adhalleran/QS. All sequence files for constructs used in this manuscript are also available at github.com/adhalleran/QS.
Supporting Table 1:
Sequences used in this study. All .gb files are available here: [https://github.com/adhalleran/QS/tree/master/Sequences](https://github.com/adhalleran/QS/tree/master/Sequences) and via email upon request.

J23106 is part of the Anderson promoter library. BCD2 was selected from the library generated by Mutalik et al., and ECK120033736 was picked from Chen et al.1,2

Highlighted regions correspond to the promoter, 5'UTR, CDS, and terminator respectively. Unhighlighted regions are scars generated by Type IIS restriction enzyme-mediated assembly.

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References


Supporting Figure 1:
TX-TL time traces from LuxR (2nM DNA) + pLux-GFP (4nM DNA) across a range of Lux AHL concentrations. Solid lines represent the mean of four replicates, shading represents +/- one standard deviation.

Supporting Figure 2:
TX-TL time traces from LuxR (2nM DNA) + pLux-GFP (4nM DNA) across a range of Las AHL concentrations. Solid lines represent the mean of four replicates, shading represents +/- one standard deviation.
Supporting Figure 3:
TX-TL time traces from LuxR (2nM DNA) + pLux-GFP (4nM DNA) across a range of Rpa AHL concentrations. Solid lines represent the mean of four replicates, shading represents +/- one standard deviation.

Supporting Figure 4:
TX-TL time traces from LuxR (2nM DNA) + pLas-GFP (4nM DNA) across a range of Lux AHL concentrations. Solid lines represent the mean of four replicates, shading represents +/- one standard deviation.
Supporting Figure 5:
TX-TL time traces from LuxR (2nM DNA) + pLas-GFP (4nM DNA) across a range of Las AHL concentrations. Solid lines represent the mean of four replicates, shading represents +/- one standard deviation.

Supporting Figure 6:
TX-TL time traces from LuxR (2nM DNA) + pLas-GFP (4nM DNA) across a range of Rpa AHL concentrations. Solid lines represent the mean of four replicates, shading represents +/- one standard deviation.
Supporting Figure 7:
TX-TL time traces from LuxR (2nM DNA) + pRpa-GFP (4nM DNA) across a range of Lux AHL concentrations. Solid lines represent the mean of four replicates, shading represents +/- one standard deviation.

Supporting Figure 8:
TX-TL time traces from LuxR (2nM DNA) + pRpa-GFP (4nM DNA) across a range of Las AHL concentrations. Solid lines represent the mean of four replicates, shading represents +/- one standard deviation.
Supporting Figure 9: 
TX-TL time traces from LuxR (2nM DNA) + pRpa-GFP (4nM DNA) across a range of Rpa AHL concentrations. Solid lines represent the mean of four replicates, shading represents +/- one standard deviation.

Supporting Figure 10: 
TX-TL time traces from LasR (2nM DNA) + pLux-GFP (4nM DNA) across a range of Lux AHL concentrations. Solid lines represent the mean of four replicates, shading represents +/- one standard deviation.
Supporting Figure 11: TX-TL time traces from LasR (2nM DNA) + pLux-GFP (4nM DNA) across a range of Las AHL concentrations. Solid lines represent the mean of four replicates, shading represents +/- one standard deviation.

Supporting Figure 12: TX-TL time traces from LasR (2nM DNA) + pLux-GFP (4nM DNA) across a range of Rpa AHL concentrations. Solid lines represent the mean of four replicates, shading represents +/- one standard deviation.
Supporting Figure 13:
TX-TL time traces from LasR (2nM DNA) + pLas-GFP (4nM DNA) across a range of Lux AHL concentrations. Solid lines represent the mean of four replicates, shading represents +/- one standard deviation.

Supporting Figure 14:
TX-TL time traces from LasR (2nM DNA) + pLas-GFP (4nM DNA) across a range of Las AHL concentrations. Solid lines represent the mean of four replicates, shading represents +/- one standard deviation.
Supporting Figure 15:
TX-TL time traces from LasR (2nM DNA) + pLas-GFP (4nM DNA) across a range of Rpa AHL concentrations. Solid lines represent the mean of four replicates, shading represents +/- one standard deviation.

Supporting Figure 16:
TX-TL time traces from LasR (2nM DNA) + pRpa-GFP (4nM DNA) across a range of Lux AHL concentrations. Solid lines represent the mean of four replicates, shading represents +/- one standard deviation.
Supporting Figure 17: TX-TL time traces from LasR (2nM DNA) + pRpa-GFP (4nM DNA) across a range of Las AHL concentrations. Solid lines represent the mean of four replicates, shading represents +/- one standard deviation.

Supporting Figure 18: TX-TL time traces from LasR (2nM DNA) + pRpa-GFP (4nM DNA) across a range of Rpa AHL concentrations. Solid lines represent the mean of four replicates, shading represents +/- one standard deviation.
Supporting Figure 19:
TX-TL time traces from RpaR (2nM DNA) + pLux-GFP (4nM DNA) across a range of Lux AHL concentrations. Solid lines represent the mean of four replicates, shading represents +/- one standard deviation.

Supporting Figure 20:
TX-TL time traces from RpaR (2nM DNA) + pLux-GFP (4nM DNA) across a range of Las AHL concentrations. Solid lines represent the mean of four replicates, shading represents +/- one standard deviation.
Supporting Figure 21:
TX-TL time traces from RpaR (2nM DNA) + pLux-GFP (4nM DNA) across a range of Rpa AHL concentrations. Solid lines represent the mean of four replicates, shading represents +/- one standard deviation.

Supporting Figure 22:
TX-TL time traces from RpaR (2nM DNA) + pLas-GFP (4nM DNA) across a range of Lux AHL concentrations. Solid lines represent the mean of four replicates, shading represents +/- one standard deviation.
Supporting Figure 23:
TX-TL time traces from RpaR (2nM DNA) + pLas-GFP (4nM DNA) across a range of Las AHL concentrations. Solid lines represent the mean of four replicates, shading represents +/- one standard deviation.

Supporting Figure 24:
TX-TL time traces from RpaR (2nM DNA) + pLas-GFP (4nM DNA) across a range of Rpa AHL concentrations. Solid lines represent the mean of four replicates, shading represents +/- one standard deviation.
Supporting Figure 25:
TX-TL time traces from RpaR (2nM DNA) + pRpa-GFP (4nM DNA) across a range of Lux AHL concentrations. Solid lines represent the mean of four replicates, shading represents +/- one standard deviation.

Supporting Figure 26:
TX-TL time traces from RpaR (2nM DNA) + pRpa-GFP (4nM DNA) across a range of Las AHL concentrations. Solid lines represent the mean of four replicates, shading represents +/- one standard deviation.
Supporting Figure 27:
TX-TL time traces from RpaR (2nM DNA) + pRpa-GFP (4nM DNA) across a range of Rpa AHL concentrations. Solid lines represent the mean of four replicates, shading represents +/- one standard deviation.

Supporting Figure 28:
Figure 1a rendered with a divergent color map rather than the sequential shown in 1a.
Supporting Figure 29: Titration of RFP DNA causes a linear increase in reporter expression. Fluorescence for each replicate was calculated as the 95th percentile value obtained across the time course. Each dot is a single replicate. Numbers above each set of four replicates is the mean of the Log2 fluorescence.

Supporting Figure 30: Crosstalk heatmap from figure 1b, shown at three different AHL concentrations ((a)100nM, (b)1uM, and (c)10uM).

Supporting Figure 31: Crosstalk heatmap from figure 1c, shown at three different AHL concentrations ((a)100nM, (b)1uM, and (c)10uM).
Supporting Figure 32: Crosstalk heatmap from figure 1d, shown at three different AHL concentrations ((a)100nM, (b)1uM, and (c)10uM).

Supporting Figure 33: (a) LasR titration with pLux at 1uM AHL. Relative fluorescence calculated at each concentration of LasR DNA (1-16nM) as 95th percentile fluorescence / mean of three pLas-GFP 95th percentile fluorescence at the same concentration of LasR. (E) LasR titration with pRpa.