1. Rehydrate bacteria.

2. Mix and incubate at 37°C overnight.

3. Re-inoculate 5 mL of bacterial suspension in new growth medium.

4. Prepare Plate A during bacterial suspension incubation by performing serial dilutions for all samples. Plate A will also include all controls (positive, negative, solvent) and blanks. After incubation, dispense 70 µL of bacterial suspension to all appropriate wells.

5. Prepare Plate B during Plate A incubation. Add 270 µL growth medium to each well. After incubation transfer 30 µL from each well of Plate A to the corresponding well in Plate B.

6. Measure absorption at 600 nm ± 20 nm. Incubate Plate B at 37°C for 2 hours.

7. Measure absorption at 600 nm ± 20 nm.

8. Prepare Plate C during Plate B incubation. Add 120 µL reagent I to each well. After incubation transfer 30 µL from each well of Plate B to the corresponding well in Plate C. Add 30 µL chromogen to each well.

9. Add 120 µL reagent L to all wells.

10. Measure absorption at 420 nm ± 20 nm.

11. Analyze results using EBPI’s bioinformatics spreadsheet.