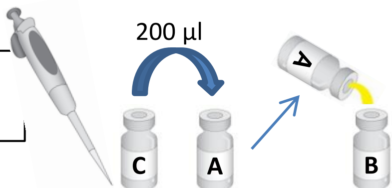

1. Rehydrate bacteria.
200 µl
C
A
B

Mix and incubate at 37°C overnight
2. Measure absorption of 600 nm ± 20 nm light. The absorption (OD) should be ≈ 0.5.
3. Re-inoculate 5 mL of bacterial suspension in new growth medium.

1
2
3
4
5
6
A
C
D
E
F
K
solvent
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.

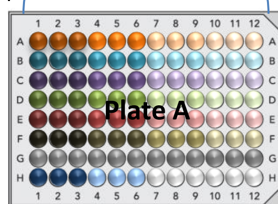
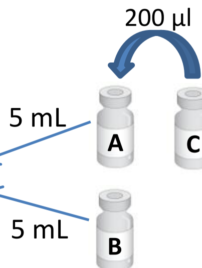



Plate A
Add 70 µL to all appropriate wells
Incubate at 37°C for 1.5 hours

200 µl
5 mL
5 mL
A
C
A
B
Incubate Plate A at 37°C for 2 hours
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.
200 µl
Add 270 µL to each well
C
A

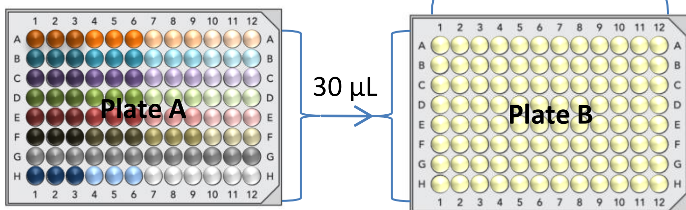



Plate A
30 µL
Plate B

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.
35 µL
Add 120 µL to all wells

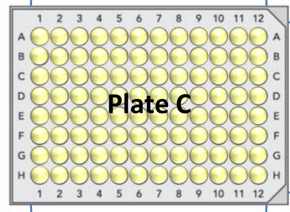



Plate C
30 µL

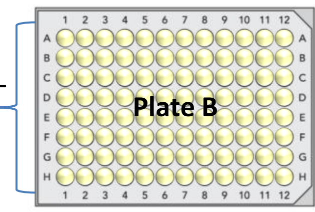
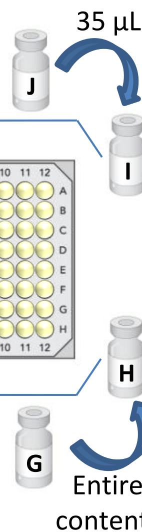



Plate B

J
I
H
G
Entire contents
Add 30 µL to all wells
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.
Incubate Plate C at 37°C for 30 minutes

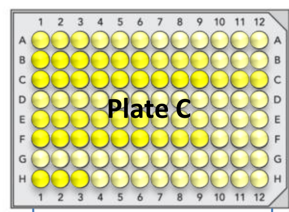



Plate C
L
9. Add 120 µL reagent L to all wells
10. Measure absorption at 420 nm ± 20 nm.

11. Analyze results using EBPI's bioinformatics spreadsheet.