# Experimenting with Copper Plates

**Purpose**

The growth of BL21 cells are tested on mueller hinton agar (MHA) plates with varying concentrations of CuSO4, dilutions of cells, and antibiotics to test for **copper toxicity, minimum inhibitory concentrations (MIC), and interactions between the media components**. This informs how the media components affect bacteria growth and the expression of the biosensor and selection genes. The goal is to determine an optimal concentration for these media components for expressing the biosensor and selection assay markers.

**Iteration 1: CuSO4 Mueller Hinton Plates without Antibiotics**

Plates were made using 38g/L MHA in MilliQ water.After microwaving, sterile CuSO4 was added to make 0mM, 0.25mM, 0.5mM, 1mM, 2mM, 4mM, and 8mM plates. Competent BL21 cells were scraped off and liquid cultured overnight. The cells were washed with saline and spotted onto the plate at different dilutions.

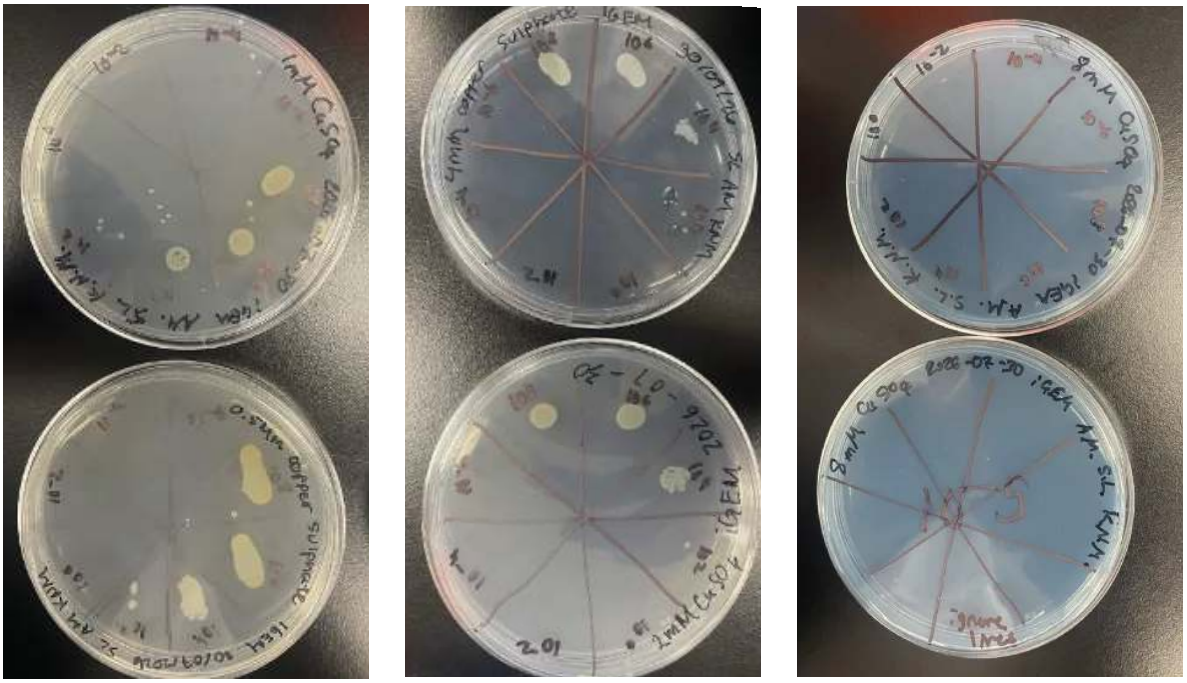


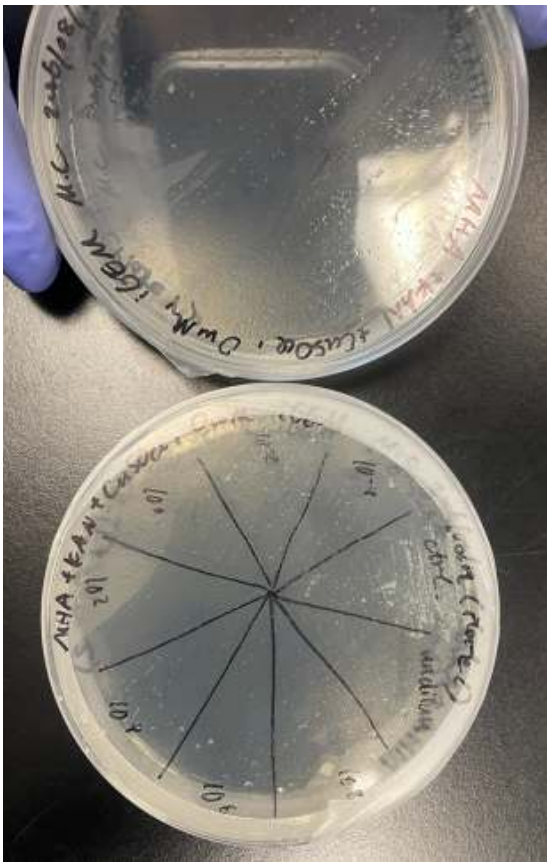
Figure 1. Copper plates with BL21 growth (1mM, 4mM, 8mM from left to right).

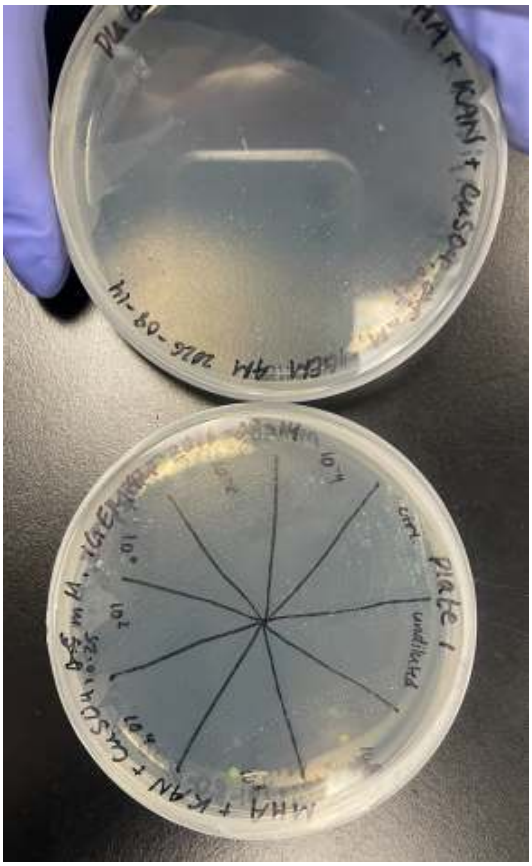
Growth is seen on all plates up to and including the 4mM CuSO4 plate for dilutions of $10^8$ to $10^2$ dilutions. No growth is observed on the 8mM plate, indicating that **the lethal concentration of CuSO4 is between 4mM and 8mM**. No growth is observed at dilutions past the $10^2$.

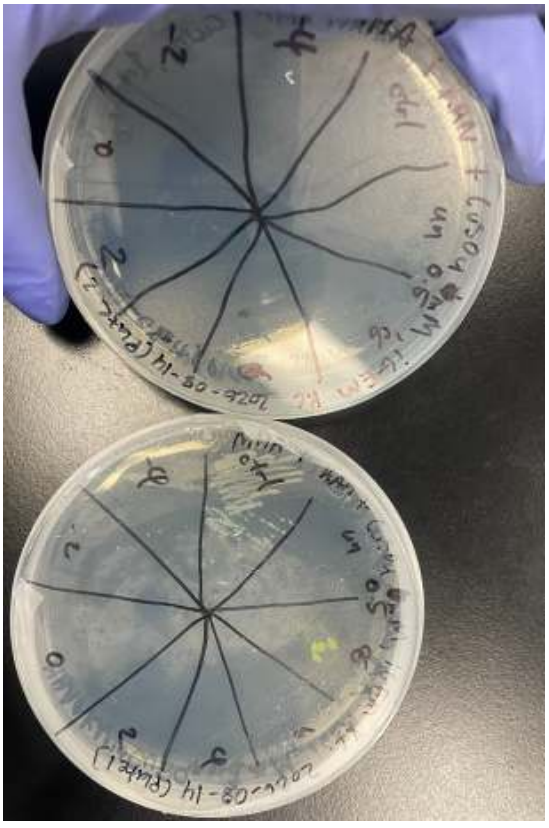
**Iteration 2: CuSO4 +kanamycin MHA plates (BIOSENSOR AS-msGFP2)**

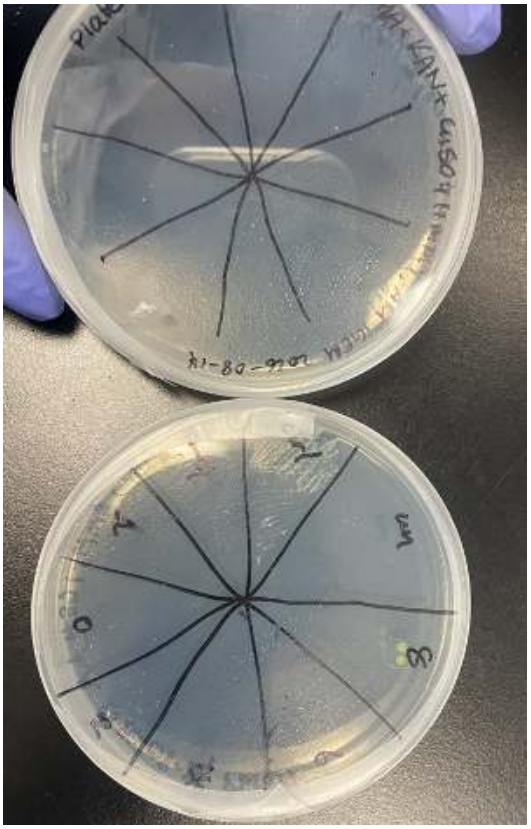
Plates were made using 38g/L MHA in MilliQ water.After microwaving, sterile CuSO4 was added to make 0mM, 0.25mM, 0.5mM, 1mM, 2mM, 4mM, and 8mM plates. Kanamycin was kept at

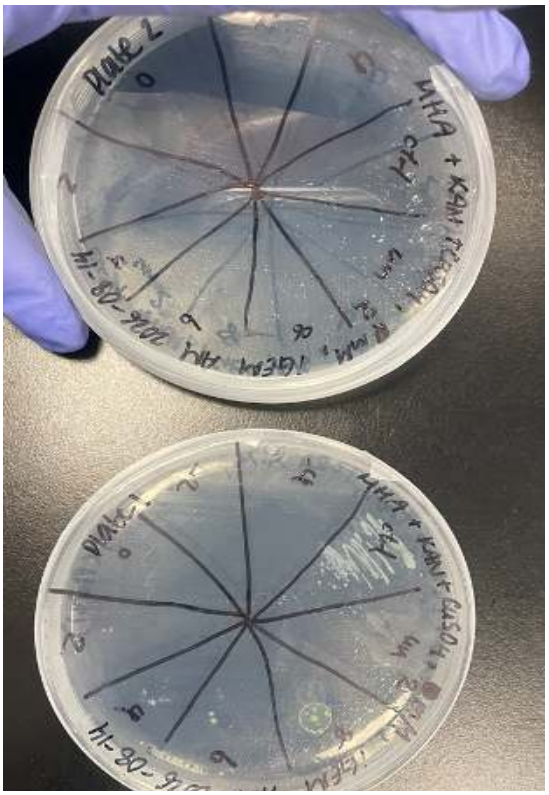
50ug/mL. BL21 transformed with AS-msGFP2 was liquid cultured overnight. The cells were washed with saline and spotted onto the plate at different dilutions (dilutions deviate from protocol, see images below).

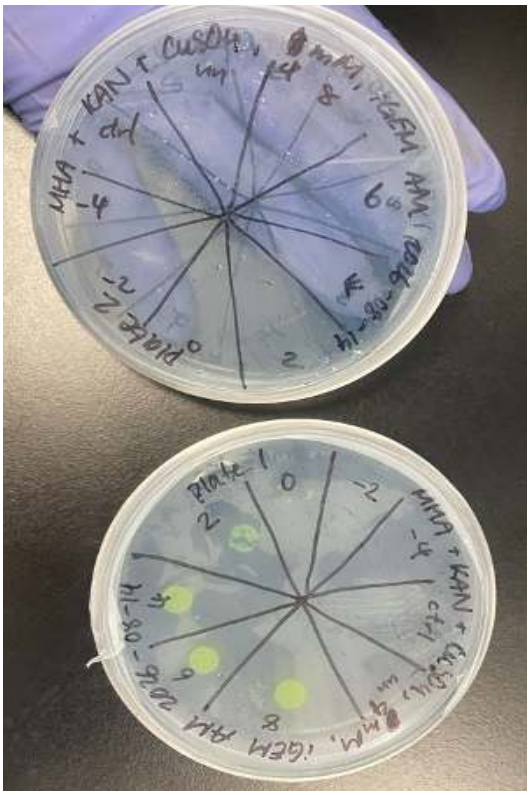


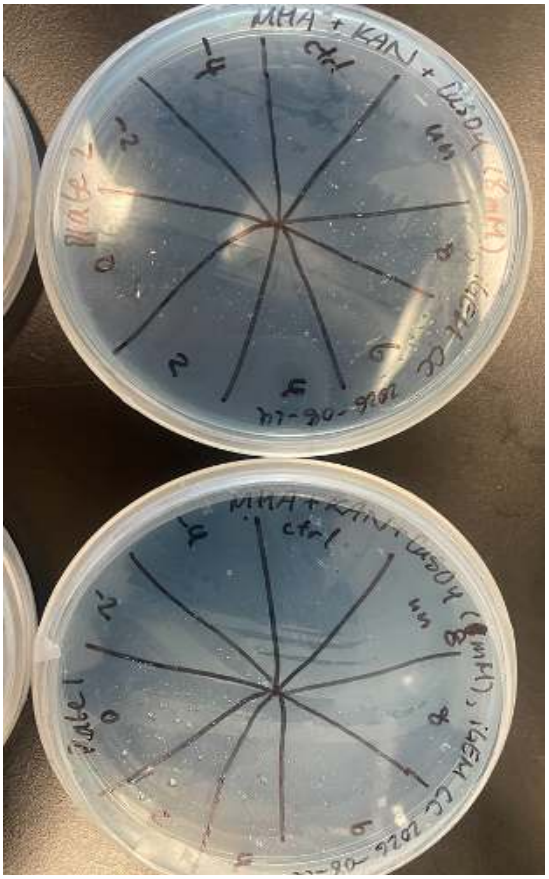


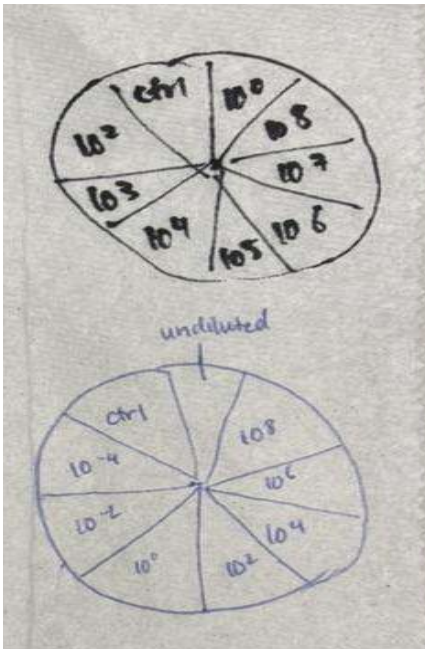










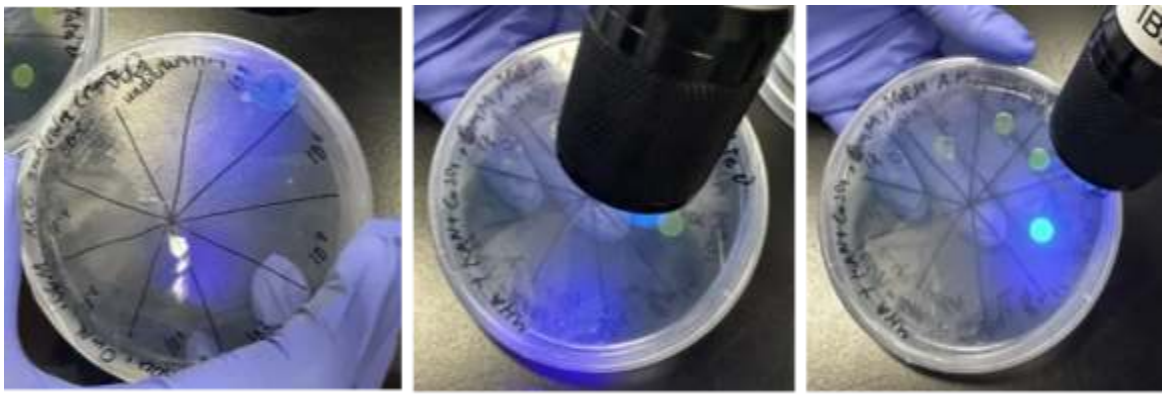



This is the actual plate layout and the actual cell dilutions used.

This was the intended plate layout and the intended cell dilutions. Map these onto the actual positions when looking at these images.

Figure 2. The growth of BL21 transformed with AS-GFP2 on MHA plates with kanamycin and different copper concentrations.

Growth is seen at the $10^8$ dilution and for CuSO4 concentrations of 4mM or less. However, the dilutions at which no growth is observed is smaller. This could be due to synergetic activities between copper and kanamycin, since kanamycin is an added variable in this iteration. However, this does not explain the outlier results at 4mM CuSO4, for which growth is observed up until the $10^0$ dilution. More testing on different dilutions is needed to determine a consistent effect of cell density.



(a) (b) (c)

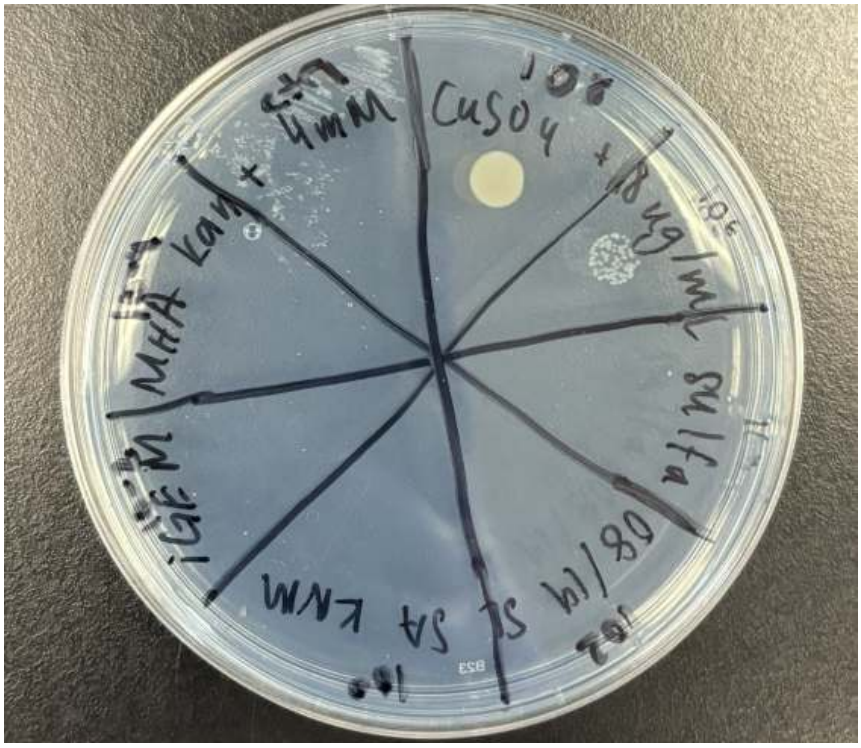
Figure 3. Select spots of growth were placed under blue light to check for fluorescence.

One negative control is growing BL21 transformed with AS-msGFP2 on a plate with 0mM CuSO4 to check if fluorescence is due to other factors than the copper-induced expression of GFP. Figure 3a shows the negative control under blue light, verifying that fluorescence is dependent on copper.

Another negative control is growing BL21 that is transformed with the empty AS plasmid with no msGFP2 on a plate with CuSO4 to check if fluorescence can be induced by copper or other factors without the expression of a fluorescent gene. Figure 3b shows the negative control under blue light, verifying that fluorescence is due to expression of the msGFP2 gene.

Figure 3c (4mM of CuSO4) shows that when copper is present to induce expression of the msGFP2 gene, the cells fluoresce under blue light. **This verifies for the biosensor assay that copper can induce GFP expression.** The next steps are to test whether GFP can be sufficiently expressed with the copper amounts that can be realistically released from the mesh.

**Iteration 3: CuSO4 + kanamycin + sulfadiazine MHA plates (SELECTION AS-sul1)**

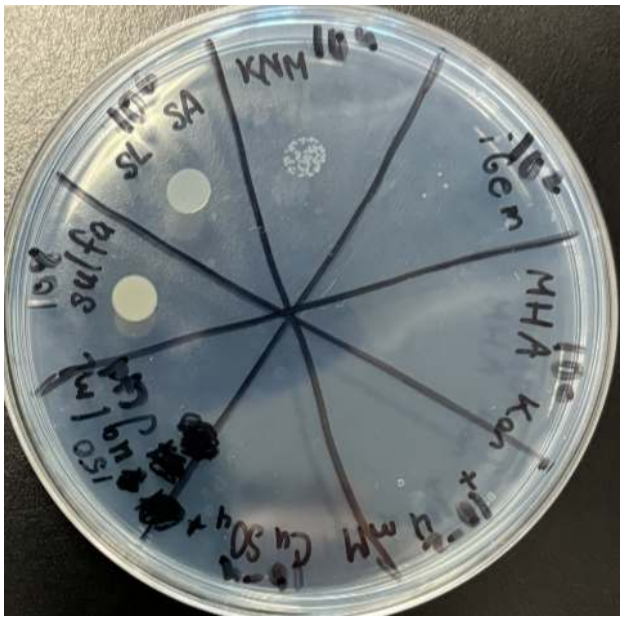



50ug/mL kan + 4mM CuSO4 + 8ug/mL sulfadiazine
Empty AS transformant grew, either sulfadiazine concentration is too low to inhibit growth or




50ug/mL kan + 4mM CuSO4 + 0ug/mL sulfadiazine
Empty AS transformant grew, as expected due to lack of sulfadiazine






50 ug/mL kan + 4mM CuSO4 + 16/64/250 sulfadiazine
Empty AS transformant did not grow, but AS-sul1 transformants grew

- 16ug/mL is threshold for killing cells without sul1 expression
- Slight decrease in growth increasing sulfa conc to 250 (10^4 dilution)
- Need 0 mM copper test to see if all cells (sul1 or not) die, to confirm promoter works

**Iteration 4: coming soon!**

**Minimum copper conc needed is between 0.125mM and 0.0625mM**