Setup

- 15 overnight cultures started on Friday oct 27th - 3 biological replicates of: Agrobacterium with either (1) pCambia, (2) pCambiaCas, (3) pGuideN1, (4) pGuideB1, (5) pGuideBC1

-On Oct 29: overnights were diluted 1/10 and then read on spec. We used these readings to dilute each of them to 0.100 OD, so now we have 15 cultures (5 different constructs, 3 biological replicates) at 0.100 OD

-Each culture was then split in to 4 different antibiotic treatments: (1) Kanamycin, (2)Kanamycin + ATC, (3) Kanamycin + Gentamycin, (4) Kanamycin + Gentamycin + ATC

-Now we have 60 tubes (5 constructs, 3 biological replicates = 15 biological conditions, each split in to 4 different treatments = 60)

Assay starts now

OD readings

At t=0, each tube had 2 mL of culture at 0.100 OD. They were grown at 30C shaking 220rpm

- at t=4, t=13, t=24hr, every culture's OD was read simultaneously on a 96 well plate reader

Plating

-Each of the 15 cultures (3 bio x 5 constructs) were diluted from 0.100 OD to 0.01 OD, and 0.001 OD.

-5 uL of each dilution was spotted on a plate in technical triplicates, on either Kan + Gent plates or Kan + Gent + ATC

-Plates were allowed to settle for 30 minutes and then incubated at 30C for two days