# CRISPR Toxicity Assay

## Overnight incubation – n=3

Inoculate a 5 mL overnight culture at 30°C of A. tumefaciens GV3101 with pCambiaCas.

## Preparation

1. Label 30 tubes according to the following:

| | | | | |
|---|---|---|---|---|
| N1, 1 hr no plasmid | N1, 2 hr no plasmid | N1, 4 hr no plasmid | N1, 8 hr no plasmid | N1, 24 hr no plasmid |
| N2, 1 hr no plasmid | N2, 2 hr no plasmid | N2, 4 hr no plasmid | N2, 8 hr no plasmid | N2, 24 hr no plasmid |
| N3, 1 hr no plasmid | N3, 2 hr no plasmid | N3, 4 hr no plasmid | N3, 8 hr no plasmid | N3, 24 hr no plasmid |
| N1, 1 hr 0.5x | N1, 2 hr 0.5x | N1, 4 hr 0.5x | N1, 8 hr 0.5x | N1, 24 hr 0.5x |
| N2, 1 hr 0.5x | N2, 2 hr 0.5x | N2, 4 hr 0.5x | N2, 8 hr 0.5x | N2, 24 hr 0.5x |
| N3, 1 hr 0.5x | N3, 2 hr 0.5x | N3, 4 hr 0.5x | N3, 8 hr 0.5x | N3, 24 hr 0.5x |
| N1, 1 hr 1.5x | N1, 2 hr 1.5x | N1, 4 hr 1.5x | N1, 8 hr 1.5x | N1, 24 hr 1.5x |
| N2, 1 hr 1.5x | N2, 2 hr 1.5x | N2, 4 hr 1.5x | N2, 8 hr 1.5x | N2, 24 hr 1.5x |
| N3, 1 hr 1.5x | N3, 2 hr 1.5x | N3, 4 hr 1.5x | N3, 8 hr 1.5x | N3, 24 hr 1.5x |

## Day of

1. Dilute overnight culture 1/10 and spec it. Calculate dilutions required to achieve 0.100 abs.
2. Dilute culture according to calculation from above to achieve a total of 2 separate 35 mL cultures (minimum) that should be OD 0.100.
    a. Culture 1: Agro grown in LB + Kan + Rif
    b. Culture 2: Agro grown in LB + Kan + Rif + 1x Anhydrous tetracycline.
3. Split each of these cultures in to 15 sterile culture tubes, and 1 mL from the remainder of each into a cuvette.
4. Take spec reading of the remainder (this will be baseline).
5. Incubate tubes on shaker at 30°C, 220 rpm.
6. At t=2 hr, 4 hr, 8 hr, 24hr, remove designated tube from the 30°C room and spec them. Record the value.

| **Overnight incubation** | | | | Do we have? |
|---|---|---|---|---|
| 1 colony | **A. tumefaciens GV3101 w/ pCambiaCas** | → | 4°C room | Y |
| 5 mL | **LB media** | → | Bench | Y |
| 15 uL each | **Rifampicin + Kanamycin** | → | -20°C under bench | Y |
| 1 | **Test tubes** | → | Bench | Y |

| | | | | |
|---|---|---|---|---|
| **Prep** | | | | |
| 30 | **Culture tubes** | → | 4°C Fridge | Y |
| **Day of** | | | | |
| 5 mL | **overnight culture** | → | 30°C Incubator | n/a |
| 70 mL | **LB media** | → | Bench | Y |
| 65 uL | **Rifampicin + Kanamycin** | → | -20°C under bench | Y |
| 35 uL | **Anhydrous tetracycline stock** | → | -20°C under bench | N |
| 7, assuming we wash | **Cuvettes for spec** | → | Beside spec | Y |
| 7 | **7 Clean cuvettes** | | | |
| 1 mL | **LB media (labeled for CRISPR)** | | | |
| | **P1000** | | | |
| | **Sterile P1000 tips** | | | |
| | **Non-sterile P1000 tips** | | | |
| | **Pen and data sheet** | | | |
| | **Test tube rack** | | | |

12:45ish 1. Gather all the items you will need:

7 Clean cuvettes
LB media (labeled for CRISPR)
P1000
Sterile P1000 tips
Non-sterile P1000 tips
Pen and data sheet
Test tube rack

12:50ish 2. Put pen, data sheet, 6 cuvettes and non-sterile P1000 tips by the spec machine.

12:55ish 3. Work near a flame and add 1 mL of LB media to a cuvette, and bring it to the spec.

➔ You should now have 7 cuvettes at the spec!

1:05ish 4. Bring test tube rack to the 30°C room and collect the 6 culture tubes, bring to spec.

1:05ish 5. Don't worry about being sterile – add 1 mL of each culture tube to each cuvette

➔ make sure you know which is which!

1:10pm 6. Blank the spec with the plain LB you put in a cuvette earlier. The spec should say OD600 and you shouldn't have to change any settings

7. Read and record the OD600 value for each culture and record it on the data sheet.

8. Bring everything back to the bench - that's it!