CRISPR Toxicity Assay – October 4 2017

Preparation

1. Inoculate a 3x 5 mL overnight cultures at 30°C of A. tumefaciens GV3101 with pCambiaCas and 1x 5 mL overnight of Agrobacterium without any plasmid.
2. Label 45 tubes according to the following:

N1, 1 hr no p - unind	N1, 2 hr no p - unind	N1, 4 hr no p - unind	N1, 8 hr no p - unind	N1, 24 hr no p - unind
N2, 1 hr no p - unind	N2, 2 hr no p - unind	N2, 4 hr no p - unind	N2, 8 hr no p - unind	N2, 24 hr no p - unind
N3, 1 hr no p - unind	N3, 2 hr no p - unind	N3, 4 hr no p - unind	N3, 8 hr no p - unind	N3, 24 hr no p - unind
N1, 1 hr no p - 1x	N1, 2 hr no p - 1x	N1, 4 hr no p - 1x	N1, 8 hr no p - 1x	N1, 24 hr no p - 1x
N2, 1 hr no p - 1x	N2, 2 hr no p - 1x	N2, 4 hr no p - 1x	N2, 8 hr no p - 1x	N2, 24 hr no p - 1x
N3, 1 hr no p - 1x	N3, 2 hr no p - 1x	N3, 4 hr no p - 1x	N3, 8 hr no p - 1x	N3, 24 hr no p - 1x
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 2x	N1, 2 hr 2x	N1, 4 hr 2x	N1, 8 hr 2x	N1, 24 hr 2x
N2, 1 hr 2x	N2, 2 hr 2x	N2, 4 hr 2x	N2, 8 hr 2x	N2, 24 hr 2x
N3, 1 hr 2x	N3, 2 hr 2x	N3, 4 hr 2x	N3, 8 hr 2x	N3, 24 hr 2x

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 3 separate 35 mL cultures (minimum) that should be OD 0.100.
    a. Culture 1: Agro (no p - 1x) grown in LB + rif
    b. Culture 1: Agro (no p - 1x) grown in LB + rif + 1x Anhydrous tetracycline
    c. Culture 2: Agro w/ plasmid grown in LB + Kan + Rif + 0.5x Anhydrous tetracycline.
    d. Culture 3: Agro w/ plasmid grown in LB + Kan + Rif + 2x 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=1hr, 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?
2 colonies	**A. tumefaciens GV3101 w/ pCambiaCas**	→	4°C room	Y
1 colony	**A. tumefaciens GV3101**	→	4°C room	Y
15 mL	**LB media**	→	Bench	Y
30 uL each	**Rifampicin + Kanamycin**	→	-20°C under bench	Y
3	**Test tubes**	→	Bench	Y
Prep				
45	**Culture tubes**	→	4°C Fridge	Y
Day of				
5 mL x3	**overnight cultures**	→	30°C Incubator	n/a
105 mL	**LB media**	→	Bench	Y
65 uL	**Rifampicin + Kanamycin**	→	-20°C under bench	Y
70 uL	**Anhydrous tetracycline stock**	→	-20°C under bench	Y
10	**Cuvettes for spec**	→	Beside spec	Y

~30 min b4 — 1. Gather all the items you will need:
- 14 Clean cuvettes
- LB media (labeled for CRISPR)
- P1000
- Sterile P1000 tips
- Non-sterile P1000 tips
- Pen and data sheet
- Test tube rack

~20 min b4 — 2. Put pen, data sheet, 13 cuvettes and non-sterile P1000 tips by the spec machine.

~15 min b4 — 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 14 cuvettes at the spec!

~5 min b4 — 4. Bring test tube rack to the 30°C room and collect the 13 culture tubes, bring to spec.

~5 min b4 — 5. Don't worry about being sterile – add 1 mL of each culture tube to each cuvette
- ➔ make sure you know which is which!

0 min — 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!