Circuit Assay Protocol

- 1 gRNA:
  - Only tet (12, 30 ng/ul)
  - Only lac (10, 100 uM)
  - Both tet and lac (low/high; high/low)
  - Tet → 3 hrs, dilute, switch to lac
  - Lac → 3 hrs, dilute, switch to tet
- 2 gRNAs:
  - Only tet (12, 30 ng/ul)
  - Only lac (10, 100 uM)
  - Both at same time (low/high; high/low)
  - Lac → 3 hrs, dilute, add lac & tet
  - Tet → 3 hrs, dilute, add lac & tet
  - Lac → 3 hrs, dilute, switch to tet
  - Tet → 3 hrs, dilute, switch to lac

1. Grow 5mL overnight cultures of cells with one of the plasmids (either the one with 1 gRNA or the one with 2 gRNAs) and of a control (pSB1C3) in 37˚C incubator.
2. The next morning, dilute the cultures to an OD_{600} of 0.05 with 12mL fresh LB with the corresponding antibiotic (spectinomycin & chloramphenicol). Place in 37˚C incubator.
3. Wait 2-3 hours until the cultures have divided several times (OD600 ~0.3-0.4). Induce with the different concentrations of the inducers listed above. (<u>below are for 10mL cultures</u>)
   a. **10uM IPTG**: 10ul of 100x dilution of 1M IPTG stock
   b. **100uM IPTG**: 10ul of 10x dilution of 1M IPTG stock
   c. **12ng/mL ATc**: 6ul of 20ug/mL ATc stock
   d. **30ng/mL ATc**: 15ul of 20ug/mL ATc stock
4. Add 200ul of the samples of these cultures into a 96-well plate to put into the plate reader. Place water on the edges of the plate. Have the plate reader measure absorbance, GFP fluorescence, and RFP fluorescence.

	1 gRNA			2 gRNAs			pSB1C3 control				
	1	2	3	4	5	6	7	8	9	10	11	12
A												
B		only tet low (12ng/ul)			only tet low (12ng/ul)			only tet low (12ng/ul)			CONTROL	
C		only tet high (30ng/ul)			only tet high (30ng/ul)			only tet high (30ng/ul)				
D		only lac low (10 uM)			only lac low (10 uM)			only lac low (10 uM)				
E		only lac high (100 uM)			only lac high (100 uM)			only lac high (100 uM)			LB	
F		tet low, lac high			tet low, lac high			tet low, lac high				
G		tet high, lac low			tet high, lac low			tet high, lac low				
H												

* We have collected data two ways: by adding 200ul of the cultures straight into the plate, and also by diluting one sample from the culture. You can do either way.

5. Place remaining cultures back in incubator for another 3 hours. After 3 hours have passed, measure the OD_{600} of the cultures. Induce the cultures that have already been induced with high concentrations of inducer with the correct amount of inducer (I just put in however much I needed for 10mL since it was about 10mL anyways).

6. Dilute it in the well to about the starting OD that the first plate had. Then, take out the plate from earlier and add 200ul of the diluted and induced samples to the corresponding wells.

1gRNA - Tet	1gRNA - Tet-->Lac	pSB1C3 - Tet-->Lac	**pSB1C3**
1gRNA - Lac	1gRNA - Lac-->Tet	pSB1C3 - Lac-->Tet	
2gRNA - Tet	2gRNA - Tet-->Tet&Lac	pSB1C3 - Tet-->Tet&Lac	
2gRNA - Lac	2gRNA - Lac-->Tet&Lac	pSB1C3 - Lac-->Lac&Tet	**LB**
pSB1C3 - Tet	2gRNA - Tet-->Lac		
pSB1C3 - Lac	2gRNA - Lac-->Tet&Lac		

7. Let the plate reader run overnight with similar settings to the first plate.