Small-molecule Induction

Colonies for induction were selected based on colony PCR results.
5uL of diluted colony (1 colony in 10uL NFW) was inoculated and incubated at 37°C / 250rpm overnight in 3.5mL minimal media with appropriate antibiotic(s).

Type of minimal media was selected based on the strain of *E. coli* being used:

Strain	**Media**	**Notes**
5alpha	M9 glycerol + Thiamine	1mM Thiamine (light-sensitive)
BL21	M9 glycerol	
10beta	M9 glycerol + Leucine	10ug Leucine/ mL (light-sensitive)
LG3.300	M9 glycerol	This strain is specific to the Synthetic Enhancer project

Note: Low-concentration antibiotics were added to inoculations in minimal media
1ul Kan/Tet per mL media
0.3uL Chlor per mL media
0.5uL Amp per mL media

Once cultures reached midlog (approximately 16 hours for inoculations in minimal media), 250uL of culture was added to a new tube; one tube per step of induction (aTc and IPTG inductions were conducted over 14 steps, arabinose inductions were conducted over 6 steps).

aTc Induction (for non-Synthetic Enhancer parts)

Make 20,000 ng/mL aTc:
Create a 1:100 dilution of stock (2mg/mL) aTc in the appropriate media + antibiotic(s)
Make 200 ng/mL aTc:
Create a 1:100 dilution of 20,000 ng/mL aTc in the appropriate media + antibiotic(s)

1. Add 250 uL of 20000 ng/mL aTc to 250 ul diluted culture to make 10000 ng/mL aTc
2. Add 125 uL of 20000 ng/mL aTc to 250 ul diluted culture to make 5000 ng/mL aTc
3. Add 50 uL of 20000 ng/mL aTc to 250 ul diluted culture to make 2000 ng/mL aTc
4. Add 25 uL of 20000 ng/mL aTc to 250 ul diluted culture to make 1000 ng/mL aTc
5. Add 12.5 uL of 20000 ng/mL aTc to 250 ul diluted culture to make 500 ng/mL aTc

6. Add 5 uL of 200000 ng/mL aTc to 250 ul diluted culture to make 200 ng/mL aTc
7. Add 250 uL of 200 ng/mL aTc to 250 ul diluted culture to make 100 ng/mL aTc
8. Add 125 uL of 200 ng/mL aTc to 250 ul diluted culture to make 50 ng/mL aTc
9. Add 50 uL of 200 ng/mL aTc to 250 ul diluted culture to make 20 ng/mL aTc
10. Add 25 uL of 200 ng/mL aTc to 250 ul diluted culture to make 10 ng/mL aTc
11. Add 12.5 uL of 200 ng/mL aTc to 250 ul diluted culture to make 5 ng/mL aTc
12. Add 5 uL of 200 ng/mL aTc to 250 ul diluted culture to make 2 ng/mL aTc
13. Add 2.5 uL of 200 ng/mL aTc to 250 ul diluted culture to make 1 ng/mL aTc
14. Add 0 uL of 200 ng/mL aTc to 250 ul diluted culture to make 0 ng/mL aTc

aTc inductions should be incubated at 37°C / 250rpm for 4-5 hours before measurement. Inductions may be kept under these conditions overnight if necessary.

aTc Induction of Synthetic Enhancer Parts (as per Amit et al)

Make 100 mg/mL Amp (1000X)
1.0 g Ampicillin
10 mL Millipore Water

Make 20 mg/mL Kan (1000X)
0.20 g Kanamycin
10 mL Millipore Water

Obtain three good colonies of LG3.300 52S OA + pACT-Tet OA cotransformation.
Inoculate them in 3 mL LB overnight with Kan and Amp from above.

Inoculate 5 uL of each overnight colony culture in 20 mL LB with Kan and Amp from above (they are 1000X, so add 20 uL Kan and 20 uL Amp per flask) in a 125 mL Flask at 37C, 250 rpm, until midlog

Spin down the LB cultures at midlog for 15 minutes at 13,000rpm
Remove supernatant

Resuspend each pellet in 100 mL Sigma 54 Broth with Kan and Amp from above
Sigma 54 Broth Recipe (as per Amit et al):
475mL Millipore water
1.5 mL glycerol
0.25g tryptone
2.9g NaCl

25ml 1M MgSO4

. 5ml 10x Pbs buffer 7.4 pH

Then set aside 2 mL in a glass culture tube, from each replicate
After setting aside the 2mL, add 1 mL 100 mM IPTG to each replicate

Make 20,000 ng/mL aTc:

Create a 1:100 dilution of stock (2mg/mL) aTc in the appropriate media + antibiotic(s)

Make 200 ng/mL aTc:

Create a 1:100 dilution of 20,000 ng/mL aTc in the appropriate media + antibiotic(s)

Dispense each replicate in 2 mL increments amongst 47 glass tubes, which each contain [aTC] solution already.

Add _____ uL	of ____ ng/mL aTC	to 2 mL Sigma 54 Broth to get _____ ng/mL aTC
0.5	200	0.05
0.642193132	200	0.064219313
0.824824037	200	0.082482404
1.059392663	200	0.105939266
1.360669384	200	0.136066938
1.747625066	200	0.174762507
2.244625629	200	0.224462563
2.882966325	200	0.288296632
3.702842346	200	0.370284235
4.755879846	200	0.475587985
6.108386745	200	0.610838674
7.845528028	200	0.784552803
10.07668843	200	1.007668843
12.9423602	200	1.29423602
16.62298966	200	1.662298966
21.35033958	200	2.135033958
27.42208288	200	2.742208288
35.22054657	200	3.522054657
45.23678621	200	4.523678621
0.581015068	20000	5.810150682
0.746247773	20000	7.462477725
0.958470388	20000	9.584703883
1.231046201	20000	12.31046201

1.58113883	20000	15.8113883
2.030792994	20000	20.30792994
2.608322626	20000	26.08322626
3.350093752	20000	33.50093752
4.302814396	20000	43.02814396
5.526475706	20000	55.26475706
7.098129482	20000	70.98129482
9.116740004	20000	91.16740004
11.70941563	20000	117.0941563
15.03941259	20000	150.3941259
19.31641494	20000	193.1641494
24.80973802	20000	248.0973802
31.86528671	20000	318.6528671
40.92733654	20000	409.2733654
0.525665089	2000000	525.6650885
0.675157019	2000000	675.1570189
0.867162401	2000000	867.1624009
1.113771476	2000000	1113.771476
1.430512785	2000000	1430.512785
1.83733097	2000000	1837.33097
2.35984266	2000000	2359.84266
3.030949497	2000000	3030.949497
3.892909899	2000000	3892.909899
5	2000000	5000

Let the tubes incubate at 37°C 250 rpm until they reach a steady state of growth

Measure 200 uL aliquots in the plate reader, taking OD600 and mCherry Fluorescence (580/610). Each replicate should take up 48 wells (47 inductions + 1 no-IPTG control).

IPTG Induction

Make 1 mM IPTG

Create a 1:100 dilution of stock (100mM) IPTG in appropriate media

1. Add 0uL to 250uL culture to make 0uM IPTG
2. Add 0.25uL 1mM IPTG to 250uL culture to make 1uM IPTG
3. Add 0.5uL 1mM IPTG to 250uL culture to make 2uM IPTG

4. Add 1.25uL 1mM IPTG to 250uL culture to make 5uM IPTG
5. Add 2.5uL 1mM IPTG to 250uL culture to make 10uM IPTG
6. Add 5uL 1mM IPTG to 250uL culture to make 20uM IPTG
7. Add 12.5uL 1mM IPTG to 250uL culture to make 50uM IPTG
8. Add 25uL 1mM IPTG to 250uL culture to make 100uM IPTG
9. Add 50uL 1mM IPTG to 250uL culture to make 200uM IPTG
10. Add 1.25uL 100mM IPTG to 250uL culture to make 500uM IPTG
11. Add 2.5uL 100mM IPTG to 250uL culture to make 1mM IPTG
12. Add 5uL 100mM IPTG to 250uL culture to make 2mM IPTG
13. Add 12.5uL 100mM IPTG to 250uL culture to make 5mM IPTG
14. Add 25uL 100mM IPTG to 250uL culture to make 10mM IPTG

IPTG inductions should be incubated 37°C / 250rpm for 4-5 hours before measurement

Arabinose Induction

Make 100mM Arabinose
    0.15g Arabinose
    1mL appropriate media
Make 1mM Arabinose (1:100 dilution)
    10uL 100mM Arabinose
    990uL appropriate media

1. Add 0uL to 250uL culture to make 0uM arabinose
2. Add 2.5 uL 1mM arabinose to 250uL culture to make 10uM
3. Add 2.5 uL 100mM arabinose to 250uL culture to make 1mM
4. Add 12.5 uL 100mM arabinose to 250uL culture to make 5mM
5. Add 25uL 100mM arabinose to 250uL culture to make 10mM
6. Add 50uL 100mM arabinose to 250uL culture to make 20mM

References

Amit, R., Garcia, H., Phillips, R., & Fraser, S. (2011). Building enhancers from the ground up: A synthetic biology approach. *Cell, 146*(1), 105-118. doi:http://dx.doi.org/10.1016/j.cell.2011.06.024